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ESSENTIAL HYPERTENSION: A DERANGEMENT IN CORTICOSTEROID METABOLISM?

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ABSTRACT. We have previously demonstrated a derangement in the peripheral metabolism of adrenal steroids in patients with essential hypertension. To substantiate further these findings, urinary excretion of a total spectrum of free and conjugated metabolites of cortisol was investigated in 13 patients with essential hypertension and 14 normotensive subjects, following i.v. administration of a tracer dose of [4-^{14}C]cortisol.

The results obtained revealed the following statistically significant differences between hypertensives and normotensives: The excretion of cortisol metabolites with reduced ring-A and non-reduced 20-ketone was lower in the hypertensives, whereas the excretion of the metabolites with intact ring-A, hydroxylated at C-6, or reduced at C-20, or both, was higher in the hypertensives. These results in conjunction with analogous results yielded by our recent study of the plasma concentrations of the same steroid metabolites, point to altered corticosteroid metabolism in patients with essential hypertension. Specifically, our studies provide evidence for a decreased activity of hepatic cortisol-$\triangle^4$-hydrogenase enzyme system, and increased activities of cortisol-20-reductase and 6-hydroxylase enzyme systems in these patients.

INTRODUCTION

Arterial hypertension is an extremely widespread phenomenon. In the United States alone, an estimated 24 million people suffer from hypertension. Although approximately half of them are unaware of having an elevated blood pressure, there is a 7:1 chance that they will eventually develop "vascular complications," leading to the impairment of the functioning capacity of the affected organ, and consequently, not infrequently, to death. There is now unequivocal evidence available from long-term cooperative studies, that even mildly but chronically elevated blood pressure results in a significantly shortened longevity.[1,2]

There are numerous functional or organic abnormalities which may lead to hypertension. Identifiable causes have been demonstrated in so-called "endocrine hypertension," which includes Cushing's syndrome (resulting from excessive production of adrenal glucocorticoids), primary hyperaldosteronism or Conn's syndrome (excessive mineralocorticoids), pheochromocytoma (excessive production of adrenal medullary hormones), certain enzymatic deficiencies of the adrenal cortex, and other endocrine disorders.[3] Also

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identified in a causal role is so-called "renal hypertension," resulting from such disorders in kidney function as stenosis of renal arteries, degenerative disorders of smaller renal blood vessels, some forms of renal parenchymal disease; and certain other anatomical abnormalities of large blood vessels, such as coarctation of aorta. In these types of hypertension, although in some the exact nature of the relationship between the "causative factor" and the mechanism of hypertension has not been yet elucidated, correction of the identifiable abnormality usually results in prompt lowering of blood pressure to normal range. These forms of hypertension can be thus considered "curable." Unfortunately, only about 20 percent of patients with arterial hypertension fall into this category. In the remaining 80 percent of hypertensives the etiology of the disease is unknown; therefore, this group, probably consisting of several types, has been labeled "idiopathic" or "essential" hypertension.

Very recently, several groups of investigators found that approximately one-fourth of patients with "essential" hypertension have low plasma renin activity (PRA) and have normal aldosterone levels.[4-8] These patients' blood pressures can be lowered by various maneuvers directly or indirectly interfering with adrenocortical function; moreover, their salivary sodium/potassium ratio is decreased. It has therefore been assumed that in the "low-renin hypertensive subgroup" of essential hypertension, there may well be an excessive production of a steroid with mineralocorticoid activity, either one of the known mineralocorticoids, or a new, as yet unidentified one.[9-13] Plasma levels of several adrenocortical steroids, 11-desoxycorticosterone (DOC), 18-hydroxy-11-desoxycorticosterone (18-OH-DOC), 16α, 18-dihydroxy-11-desoxycorticosterone (16α-OH-18-OH-DOC) and 16β-hydroxy-dehydroepiandrosterone (16β-OH-DHEA), or urinary excretion of their metabolites, were found by various investogators to be increased in some patients with "low-renin essential hypertension,"[14-18] but the definitive proof that these steroids are responsible for these patients' hypertension is lacking. The exchangeable sodium pool has not been shown conclusively to be increased in these patients, nor was the circulating plasma volume found to be expanded—the two prerequisites for a diagnosis of a classical "mineralocorticoid hypertension.[19] A hypothesis endeavoring to reconcile these discrepancies has been proposed.[20]

In the remaining three-fourths of patients with essential hypertension, those with normal PRA, the situation is even more enigmatic. There is no evidence for increased secretion rates of any adrenal steroids in these patients, although ample evidence exists that adrenocortical secretion is a *sine qua non* in the development and maintenance of these patients' arterial hypertension.[21]

Thus, the relation of adrenocortical hormones to the mechanism of essential hypertension remains unknown.

The results of our previous studies revealed that plasma levels[22] and urinary excretion[23] of various groups of conjugated 17-hydroxycorticosteroids in patients with essential hypertension are significantly different from those in normotensive subjects. Furthermore, study of individual C_{21} α-ketolic adrenal steroids in these subjects pinpointed specific steroid metabolites presumably responsible for these changes.[24] In order to confirm these findings with more specific methods, and to obtain more information with regard to the production rates of various peripheral metabolites of adrenal steroids in patients with essential hypertension, we have initiated a comprehensive study of a *total spectrum* of free and conjugated metabolites of various adrenocortical steroids, following intravenous administration of radioisotopically-labeled tracers of these steroids. The study of metabolism of cortisol in these patients and in normotensive subjects has been completed, and the results pertaining to plasma steroids were recently published.[25] This report describes the results of the study of urinary steroids.

MATERIALS AND METHODS

This study was carried out in 14 normotensive subjects and 13 patients with uncomplicated essential hypertension. The group of hypertensive patients consisted of seven men ranging in age from 28 to 47 years, and six women ranging in age from 25 to 62 years. Duration of the disease in this group was between 2 and 15 years. All the patients had normal renal function as judged by urinalysis, intravenous pyelogram, blood urea nitrogen levels (mean 14 mg/100 ml; range 11-22 mg/100 ml), endogenous creatinine clearance, and, in several instances, phenolsulfonphtalein excretion and radioactive hippuran scan. Liver function was normal. Urinary excretion of catecholamines (VMA) was within normal range; the phentolamine test was negative. Blood electrolytes and bicarbonate were normal, and 24-hr urinary aldosterone excretion, measured by double isotope dilution technique,[26] or by radioimmunoassay,[27] was within normal range (2.5-19 μg/24 hr). Urinary 17-hydroxycorticosteroids and 17-ketosteroids were not elevated. Blood glucose, fasting and 2-hr post-prandial, was within normal range. Chest X-ray and electrocardiogram were normal, except for a mild-to-moderate ventricular hypertrophy in nine patients. None of the patients exhibited signs of congestive heart failure. None had any detectable endocrine abnormalities. All patients had a family history of hypertension. All were off any medication for a period of at least two weeks prior to this study. The blood pressure in this group ranged between 145/94 and 230/145 mm Hg, the mean being 186/115.

The normotensive subjects consisted of eight men ranging in age from 23 to 48 years, and six women ranging in age from 24 to 57 years. They were either members of the medical school faculty or the hospital staff, or their spouses. Clinical and laboratory examinations of these subjects, which included the same tests as those carried out on the hypertensive patients, were all entirely normal. Blood pressure ranged between 105/68 and 145/87 mm Hg, the mean being 123/76.

Patients and normal control subjects were informed alike that certain medications could influence steroid enzyme systems involved and, therefore, any medication should be suspended for a period of two weeks prior to and during the study. An assurance from all subjects was obtained that they understood the importance of this study and would comply with the instruction not to take any medication, including oral contraceptives or "sleeping pills."

None of the female subjects studied was pregnant. All of those in the reproductive age had normal menstrual cycles. Because of a possible influence of estrogens secreted during the menstrual cycle on the metabolic pathway of cortisol, the collection of urine, following the administration of the tracer (see below), was carried out in the female subjects in the period between the eighth and eleventh days of the cycle.

None of the subjects was restrained in normal daily activities, but all were instructed to avoid overly strenuous work, both physical and mental. No dietary restrictions were applied.

A tracer dose of [4-^{14}C] cortisol (20 μc) was administered to each of the investigated subjects exactly at 8:00 a.m. in a rapid i.v. injection. The mode of preparation and the injection of the tracer were previously described.[25] Following administration of the tracer, urine was collected for two consecutive 24-hr periods. Radioactivity was measured in both specimens, but only the first 24-hr urine collection, which contained 85.0 ± 5.2 (S.D) percent of the administered radioactivity in the normotensives and 77.6 ± 6.3 percent in the hypertensives was used for the isolation of free and conjugated steroid metabolites. Urine was collected on ice, and frozen immediately following the completion of 24-hr collection. The frozen specimens were stored at −15°C until further processing.

The methods employed for the extraction, purification, isolation and quantita-

tion of various free and conjugated steroid metabolites were reported in detail previously.[28-34] Briefly, "free" steroid metabolites were extracted with ethyl acetate; conjugated steroid metabolites were removed by means of an Amberlite XAD-2 column. Various groups of conjugated steroids (glucuronides, sulfates, steroids complexed with nucleosides) were then separated from each other by means of high-voltage paper electrophoresis (H.V.E.)[29,35] Individual steroid metabolites present in each of these groups were then chromatographically separated either as individual steroid-conjugates, or as free steroids released by a specific hydrolytic or solvolytic cleavage of a given group of conjugates. In either case, the separated steroid metabolites were identified[25,28,30,34] and then quantitated by counting their radioactivity in a Packard liquid scintillation spectrometer.[30] All values obtained from scintillation counting were corrected for quenching, by means of especially-constructed quenching curves, using corresponding urinary extracts or chromatogram-eluates as quenchers. The reproducibility of counting was maintained with 2 percent standard deviation.

In the presentation of the results, individual steroid metabolites were grouped into: (1) metabolites with intact $\triangle^4$-3-ketone, intact side-chain, and no additional substitution groups on the steroid nucleus (cortisol, cortisone);* (2) 6-hydroxy derivatives of the latter; (3) 20-reduced metabolites of the steroids with the ring-A intact and no other substitution groups (20α- and 20β-dihydrocortisol); (4) 6-hydroxylated and 20-reduced metabolites (all four isomers) with the ring-A intact; (5) metabolites with the ring-A reduced ("tetrahydro" derivatives) and 20-ketone preserved (THF, THE, αTHF, αTHE); (6) metabolites with the ring-A and 20-ketone reduced (cortols and cortolones); (7) metabolites with the side-chain oxidized to 17-ketone (C_{19} steroids).

RESULTS

The 24-hr urinary excretion and the relative urinary concentrations of various groups of free and conjugated metabolites of cortisol in normotensives and hypertensives are shown in Table I. It will be seen that both the excretion and the concentrations of glucuronide conjugated metabolites were significantly lower in patients with essential hypertension than in normotensive subjects, while those of sulfate conjugated metabolites, and, even more so, those of *very polar* steroid metabolites, complexed with nucleosides ("N"-conjugates), were found to be markedly higher in the hypertensives. Concentrations of free steroid metabolites were also higher in the hypertensives, but their mean was statistically not significantly different from that in the normotensives. It should be

*The following abbreviations and trivial names are used for steroids and their conjugates: cortisol (Fk) for 11β,17α,21-trihydroxy-4-pregnene-3,20-dione; cortisone (Ek) for 17α,21-dihydroxy-4-pregnene-3,11,20-trione; 6α-hydroxycortisol (6α-OH-F) for 6α,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione; 6β-hydroxycortisol (6β-OH-F) for 6β,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione; 20α-dihydrocortisol (20α-DHF) for 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one; 20β-dihydrocortisol (20β-DHF) for 11β,17α,20β,21-tetrahydroxy-4-pregnen-3-one; 6β-hydroxy-20α-dihydrocortisol (6β-OH-20α-DHF) for 6β,11β,17α,20α,21-pentahydroxy-4-pregnen-3-one; 6β-hydroxy-20β-dihydrocortisol (6β-OH-20β-DHF) for 6β,11β,17α,20β,21-pentahydroxy-4-pregnen-3-one; tetrahydrocortisol (THF) for 3α,11β,17α,21-tetrahydroxypregnan-20-one; *allo*tetrahydrocortisol (*allo*THF or αTHF) for 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one; tetrahydrocortisone (THE) for 3α,17α,21-trihydroxypregnene-11,20-dione; *allo*tetrahydrocortisone (*allo*THE or αTHE) for 3α17α,21-trihydroxy-5α-pregnane-11,20-dione; α-cortol for 3α,11β,17α,20α,21-pentahydroxy-5β-pregnane; α-*allo*cortol for 3α-11β,17α,20α,21-pentahydroxy-5α-pregnane; β-cortol for 3α,11β,17α,20β,21-pentahydroxy-5β-pregnane; β-*allo*cortol for 3α,11β,17α,20β,21-pentahydroxy-5α-pregnane; α-cortolone for 3α,17α,20α,21-tetrahydroxy-5β-pregnan-11-one; α-*allo*cortolone for 3α,17α,20α,21-tetrahydroxy-5α-pregnan-11-one; β-cortolone for 3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one; β-*allo*cortolone for 3α,17α,20β,21-tetrahydroxy-5α-pregnan-11-one; 11β-hydroxy-etiocholanolone (11-OH-Etio) for 3α,11β-dihydroxy-5β-androstan-17-one; 11β-hydroxy-androsterone (11-OH-Andro) for 3α,11β-dihydroxy-5α-androstan-17-one; 11-keto-etiocholanolone (11-keto-Etio) for 3α-hydroxy-5β-androstane-11,17-dione; 11-keto-androsterone (11-keto-Andro) for 3α-hydroxy-5α-androstane-11,17-dione; -sulfate (-S) for (steroid)-yl-sulfate; -glucuronide (-G) for (steroid)-yl-β-D-glucopyranosiduronide.

TABLE I

RADIOACTIVITY AND RELATIVE CONCENTRATIONS OF VARIOUS GROUPS OF URINARY METABOLITES OF [4-^{14}C]CORTISOL IN NORMOTENSIVE SUBJECTS (N) AND PATIENTS WITH ESSENTIAL HYPERTENSION (H)

Steroid Group*	DPM (×10²)‡			% of Sum Total of All 4 Groups					
	N	H	Differ. (H−N)†	N	H	Differ. (H−N)†	% Differ.§	t	P
Gluc.	226,015 ±15,432	179,112 ±12,583	−46,903	74.7 ±4.8	61.5 ±3.9	−13.2	18 ↓	7.5115	<0.001
Sulf.	20,574 ±6,851	29,998 ±9,320	+9,424	6.8 ±1.5	10.3 ±3.2	+3.5	52 ↑	3.5407	<0.01
"N"	19,365 ±9,850	36,695 ±12,580	+17,330	6.4 ±3.1	12.6 ±4.2	+6.2	97 ↑	4.2190	<0.001
Free	36,610 ±10,890	45,433 ±11,358	+8,823	12.1 ±3.6	15.6 ±3.9	+3.5	29 ↑	2.3330	<0.05
Total	302,564	291,238	−11,326	100.0	100.0	0			

*Gluc.—glucuronide conjugates; Sulf.—sulfate conjugates; "N"—group of very polar metabolites of cortisol bound to nucleosides (presumably in a complex form), and separated from the other groups of conjugates by means of H.V.E.; Free—steroids extracted with ethyl acetate, prior to the extraction of conjugates.
‡Radioactivity of a given group of cortisol metabolites excreted during 1st 24 hours after the administration of the tracer, mean ± S.D.; all values corrected for losses incurred during extraction and H.V.E.
†Difference between mean values for H and N.
§Mean values for N taken as 100%; difference between means of H and N calculated as percent increase (↑) or decrease (↓) for means for N.

pointed out that the free (unconjugated) steroids constitute a small fraction of urinary metabolites of cortisol (10 ± 5 percent of all cortisol metabolites, as compared to 50 ± 10 percent in plasma), due to their low renal clearance.*

Investigation into the steroidal composition of the above groups of cortisol metabolites revealed the following:

(1) The bulk of glucuronide conjugated metabolites of cortisol consisted of steroids conjugated at C-3. In this group, the largest fraction (52 percent in the normotensives) consisted of steroids reduced in ring-A with 20-ketone intact—THF, THE, and their 5α-epimers (approximately ⅕ of the THF was found to be conjugated at C-21.)[30] Steroids with ring-A reduced and 20-ketone reduced, cortols and cortolones (4 isomers of each, 5α20α, 5α20β, 5β20α and 5β20β), made up the second largest fraction (23 percent in the normotensives) of glucuronide conjugates. The third largest fraction of steroids in this conjugate group consisted of ring-A reduced steroids with side-chain oxidized to 17-ketone—11-hydroxy- and 11-keto-etiocholanolone and 11-hydroxy- and 11-keto-androsterone (20 percent of all glucuronide conjugates in the normotensives). The remaining (5 percent) identified in the group of glucuronide conjugated metabolites of cortisol had all nonreduced ring-A (Δ^4-3-keto configuration preserved) and were conjugated at C-12 (cortisol, cortisone, and their 6-hydroxylated and/or 20-reduced metabolites).

(2) The bulk of sulfate conjugated metabolites of cortisol consisted of C-3 sulfates of steroids with 20-ketone reduced (cortols and cortolones) (28 percent in the normotensives), and of C-21 sulfates of steroids with Δ^4-3-keto configuration preserved, and either reduced 20-ketone (20-DHF, 6-OH-20-DHF) (14 percent)

*95 to 98 percent of plasma cortisol and cortisone are bound to corticosteroid-binding globulin (CBG, transcortin), and furthermore, about ½ of the unbound ones, found in the glomerular ultrafiltrate, are reabsorbed from the renal tubules

or intact 20-ketone (FK and EK, and their 6-hydroxy derivatives) (15 percent). Thus, steroids reduced at C-20 and/or conjugated at C-21 comprised 57 percent of all sulfate conjugated metabolites of cortisol in the normotensives (65 percent in the hypertensives). The other steroids identified in the group of sulfate conjugates were THF, THE, and their 5α-epimers (25 percent), and steroids with side-chain oxidized to 17-ketone (11-oxy-17-KS) (19 percent); all of these metabolites were found to be conjugated at C-3.

(3) The group of "N"-conjugates consisted almost entirely of very polar metabolites of cortisol hydroxylated at C-6 and reduced at C-20; 93 percent of these steroids had Δ^4-3-keto configuration preserved.

(4) The bulk (81 percent) of the group of free cortisol metabolites consisted of steroids with nonreduced ring-A: cortisol and cortisone (23 percent), and their 6-hydroxylated (33 percent), 20-reduced (13 percent), and 6-hydroxylated *and* 20-reduced (12 percent) metabolites. Steroids with ring-A reduced constituted the remainder of this group of metabolites: THF, THE and their 5α-epimers (10 percent), cortols and cortolones (3 percent), and C_{19} metabolites (steroids with side-chain oxidized to 17-ketone) (5 percent).

Table II lists relative concentrations of

TABLE II

RELATIVE CONCENTRATIONS OF VARIOUS CORTISOL METABOLITES WITHIN EACH FREE AND CONJUGATED STEROID GROUP IN URINE OF NORMOTENSIVE SUBJECTS (N) AND PATIENTS WITH ESSENTIAL HYPERTENSION (H)

Steroids*	% of Total Cortisol Metabolites Excreted over 24-hr Period									
	N					H				
	Gluc.§	Sulf.	"N"	Free	Total	Gluc.	Sulf.	"N"	Free	Total
FK+EK	1.8‡ ±0.4 (2.4)†	0.7 ±0.3 (10.3)	—	2.8 ±0.5 (23.1)	5.3 ±1.3	2.3 ±0.5 (3.7)	1.2 ±0.5 (11.6)	—	3.1 ±0.7 (19.9)	6.6 ±1.6
20-DHF (20α+20β)	0.5 ±0.3 (0.7)	0.8 ±0.2 (11.8)	—	1.6 ±0.6 (13.2)	2.9 ±1.6	0.7 ±0.4 (1.2)	1.8 ±0.4 (17.5)	—	2.5 ±0.9 (16.0)	5.0 ±2.1
6-OH-F (6α+6β)	—	0.3 ±0.2 (4.4)	—	4.0 ±1.8 (33.1)	4.3 ±2.2	—	0.5 ±0.3 (4.9)	—	5.7 ±1.9 (36.5)	6.2 ±2.4
6-OH-20-DHF (6α+6β, 20α+20β)	1.2 ±0.5 (1.6)	0.1 ±0.1 (1.5)	6.4 ±3.1	1.5 ±0.6 (12.4)	9.2 ±3.1	1.6 ±0.5 (2.6)	0.3 ±0.2 (2.9)	12.6 ±4.2	2.1 ±0.6 (13.5)	16.6 ±4.2
THF+THE (5α+5β)	38.5 ±4.4 (51.5)	1.7 ±0.3 (25.0)	—	1.2 ±0.2 (9.9)	41.4 ±4.8	29.9 ±3.8 (48.6)	1.9 ±0.2 (18.4)	—	1.0 ±0.2 (6.4)	32.8 ±3.9
cortols + cortolones	17.2 ±5.0 (23.0)	1.9 ±0.5 (27.9)	—	0.4 ±0.2 (3.3)	19.5 ±4.3	14.2 ±3.8 (23.1)	2.9 ±0.7 (28.2)	—	0.4 ±0.3 (2.6)	17.5 ±5.5
11-oxy-17-KS	15.5 ±2.5 (20.8)	1.3 ±0.5 (19.1)	—	0.6 ±0.3 (5.0)	17.4 ±2.9	12.8 ±2.8 (20.8)	1.7 ±0.3 (16.5)	—	0.8 ±0.2 (5.1)	15.3 ±3.5
Total	74.7 (100.0)	6.8 (100.0)	6.4	12.1	100.0	61.5 (100.0)	10.3 (100.0)	12.6	15.6	100.0

*For steroid abbreviations see footnote * in text.
§For designation of steroid groups see legend to Table I.
‡Mean ± S.D.
†Values in parenthesis represent mean urinary concentrations of various steroid metabolites calculated as percent of all steroids present in this group of metabolites only (glucuronides, sulfates, etc.)

the individual metabolites of cortisol within each free and conjugated steroid group, in normotensive subjects and in patients with essential hypertension.

Glucuronide-conjugates. Steroids reduced in ring-A (THF and THE; cortols and cortolones; 11-oxy-17-KS) were found to be lower in the hypertensives. Steroids with nonreduced ring-A, hydroxylated in C-6 position and/or reduced at C-20, were all higher in the hypertensives.

Sulfate conjugates. Although values for all steroids in this group were higher in the hypertensives, when they were related to the concentration of total sulfates, taken as 100 percent, they were found to follow a pattern similar to that of the metabolites in the glucuronide-conjugated steroid group.

"N"-conjugates. Concentrations of all cortisol metabolites in the group (all of them hydroxylated at C-6 and reduced at C-20) were found to be higher in the hypertensives.

"Free" steroids. The concentrations of all steroids in this group of cortisol metabolites followed the same pattern as those of the steroids in the conjugated metabolite groups.

Values for the excretion and the relative concentrations of the identical steroids from each free and conjugated group of cortisol metabolites were totaled, and the statistical significance of the differences between the means was calculated. These results are shown in Table III. It will be seen that the concentrations of steroids with the ring-A reduced and 20-ketone preserved (THF, THE, and their 5α-epimers) were very significantly lower in the hypertensives than in the normotensives (the difference in the mean was 21 percent). The steroid metabolites with both ring-A and 20-ketone reduced (cor-

TABLE III

RADIOACTIVITY AND RELATIVE CONCENTRATIONS OF VARIOUS CORTISOL METABOLITES (TOTALS OF ALL FREE AND CONJUGATED STEROID GROUPS) IN URINE OF NORMOTENSIVE SUBJECTS (N) AND PATIENTS WITH ESSENTIAL HYPERTENSION (H)

Steroids*	DPM ($\times 10^2$)‡			% of Total Cortisol Metabolites					
	N	H	Differ. (H−N)†	N	H	Differ. (H−N)†	% Differ.§	t	P
F_K+E_K	16,035 ±4,330	19,220 ±5,469	+3,185	5.3 ±1.3	6.6 ±1.6	+1.3	25 ↑	2,2362	<0.05
20-DHF (20α+20β)	8,774 ±4,852	14,562 ±6,511	+5,788	2.9 ±1.6	5.0 ±2.1	+2.1	72 ↑	2,8239	<0.01
6-OH-F (6α+6β)	13,010 ±6,865	18,056 ±6,980	+5,046	4.3 ±2.2	6.2 ±2.4	+1.9	44 ↑	2.0650	<0.05
6-OH-20-DHF (6α+6β, 20α+20β)	27,835 ±9,793	48,344 ±10,281	+20,509	9.2 ±3.1	16.6 ±4.2	+7.4	80 ↑	5.0355	<0.001
THF+THE (5α+5β)	125,260 ±15,628	95,526 ±13,158	−29,734	41.4 ±4.8	32.8 ±3.9	−8.6	21 ↓	4.8938	<0.001
cortols + cortolones	58,999 ±14,922	50,967 ±16,017	−8,032	19.5 ±4.3	17.5 ±5.5	−2.0	10 ↓	1.0166	<0.4
11-oxy-17-KS	52,645 ±9,874	44,560 ±10,193	−8,085	17.4 ±2.9	15.3 ±3.5	−2.1	12 ↓	1.6378	<0.2
Total	302,558	291,235	−11,323	100.0	100.0	0			

*For steroid abbreviations see footnote * in text.
‡Radioactivity of a given group of cortisol metabolites excreted during 1st 24 hours after the administration of the tracer, mean ± S.D.; all values corrected for losses incurred during extraction and H.V.E.
†Difference between mean values for H and N.
§Mean values for N taken as 100%; difference between means of H and N calculated as percent increase (↑) or decrease (↓) from means for N.

tols and cortolones) constituted also a smaller fraction of all cortisol metabolites in the hypertensives than in the normotensives; however, the difference between the means of their relative concentrations (10 percent) was considerably smaller (2.1 times) than that between the mean concentrations of the corresponding steroids with nonreduced 20-ketone. This difference was found to be statistically not significant. Similarly, the concentrations of steroid metabolites with the side-chain oxidized to 17-ketone (most of them reduced in ring-A) were also found to be lower in the hypertensives than in the normotensives; the difference between the means of their concentrations (12 percent) was also considerably smaller (1.8 times) than that between the mean concentrations of THF/THE-type metabolites.

In contrast, the concentrations of all steroids with the Δ^4-3-keto configuration preserved (ring-A not reduced) were found to be significantly higher in the hypertensives than in the normotensives. The highest concentrations were those of 20-reduced metabolites (72 percent increase in the mean values) and 6-hydroxylated and 20-reduced metabolites (80 percent increase). The statistical significance of the difference between the corresponding means was of a high order.

DISCUSSION

The results of this study reveal significant differences between patients with essential hypertension and normotensive subjects in the urinary excretion rates of various free and conjugated metabolites of cortisol. Since our study of the plasma concentrations of various cortisol metabolites revealed analogous differences between normotensives and hypertensives,[25] it may be deduced that the production rates of various peripheral metabolites of cortisol in patients with essential hypertension are abnormal: those of ring-A reduced steroids are significantly lower in these patients than in normotensive subjects, while those of steroid metabolites with intact ring-A, but reduced at C-20 and/or hydroxylated at C-6, are significantly higher in the hypertensives. These findings provide indirect evidence for: 1) decreased activity of cortisol ring-A reductase (Δ^4-hydrogenase) enzyme system (found in the human exclusively in the liver cells); 2) increased activities of cortisol 20-reductase and 6-hydroxylase enzyme systems (the former is present in many different tissues besides the liver, including fibroblasts and lymphocytes, the latter has been found in the adrenal and in the liver, but its occurrence in other tissues has not been investigated).

Two questions thus arise: (1) Are the detected abnormalities in the activities of cortisol metabolizing enzymes interdependently related, and, if so, what is the nature of this relationship and the time-sequence of their occurrence? (2) Are the detected abnormalities cause or effect of the elevated blood pressure in patients with essential hypertension?

With regard to the first question, two possibilities may be suggested (Fig. 1):

A. The increase in the activities of cortisol 20-reductase and/or 6-hydroxylase is the initial abnormality, which results in an increased utilization rate of cortisol substrate, and consequently, in its decreased availability for the ring-A reductase system, leading to a diminished production rate of the tetrahydrogenated metabolites, even in the presence of a fully preserved capacity of Δ^4-hydrogenase.

B. The decrease in the activity of Δ^4-hydrogenase is the initial abnormality, which results in a decreased utilization rate of cortisol substrate; consequently, there is an excess of unmetabolized cortisol within the hepatic metabolic compartment, leading to a compensatory increase in the activities of cortisol 20-reductase and 6-hydroxylase (as well as cortisol-21-glucuronyl transferase and cortisol-21-sulfokinase).

The overall decrease in the production of glucuronide conjugates and the overall increase in the production of sulfate conjugates, are evidently secondary to the decreased production of ring-A reduced me-

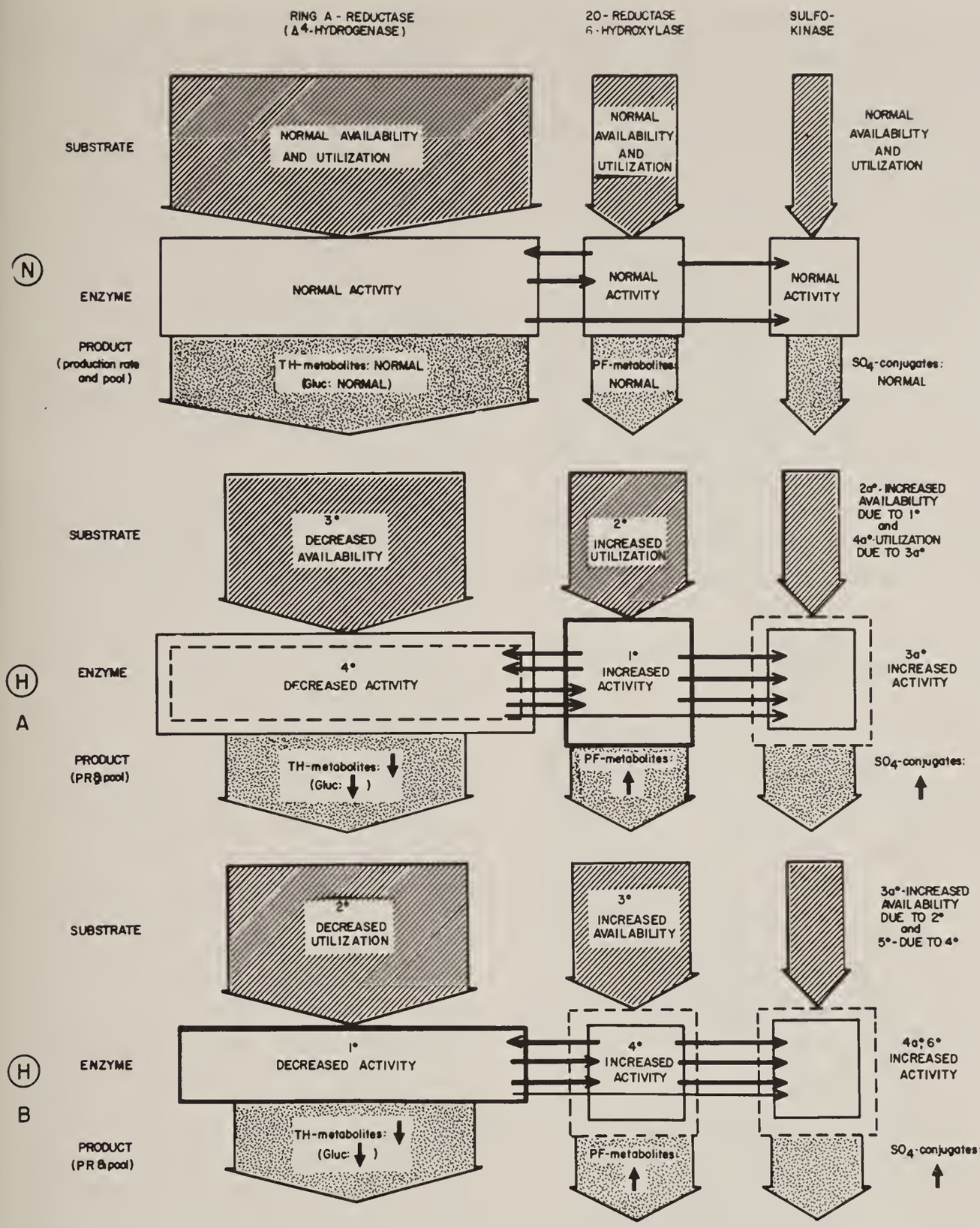


Fig. 1—DIAGRAMATIC REPRESENTATION OF INTERRELATIONSHIPS BETWEEN ACTIVITIES OF VARIOUS ENZYMES METABOLIZING CORTISOL, AVAILABILITY AND UTILIZATION OF SUBSTRATES, AND PRODUCTION RATES OF RESULTING PRODUCTS. (N): Normal subjects. (H): Hypertensive patients; A and B, two possible sequences of occurrence of the detected abnormalities. Arrows between rectangles indicate flow of products of one enzyme, from its compartment, into another's compartment for further metabolism (*e.g.*, THF, from 5β-reductase compartment, into 20β-reductase compartment, to be converted into β-cortol); the number and thickness of arrows signify increase or decrease in availability and utilization of these "secondary" substrates. Small arrows indicate increase (↑) or decrease (↓) in production rates of various cortisol metabolites. A thick-lined rectangle indicates that this enzyme's activity is the initial abnormality in the postulated sequence of metabolic events. Dotted-lined rectangle indicates a secondary increase or decrease in this enzyme's activity. Decrease or increase in size of a corresponding geometrical figure representing a substrate, an enzyme, or a product indicates decrease or increase in this substrate's availability or utilization, this enzyme's activity, or this product's production rate. 1°, 2°, 3°, etc., indicate order of postulated sequences.

tabolites, and the increased production of 20-reduced metabolites (with or without hydroxyl group at C-6), respectively. Indeed, as our investigation revealed, the majority of glucuronide conjugates consists of ring-A reduced steroids, while the majority of sulfate conjugates consists of 20-reduced metabolites (with either reduced or intact ring-A) and of 21-sulfates of ring-A nonreduced metabolites.

Recently Genest et al.,[36-38] reported abnormalities in the metabolism of aldosterone in patients with essential hypertension, which are analogous to the abnormalities in the metabolism of cortisol found by us, namely, a significantly decreased urinary excretion of ring-A reduced metabolite—tetrahydroaldosterone, and an increased excretion of the ring-A nonreduced metabolite — aldosterone-18-glucuronide. It is of interest that recently reported data indicate that, contrary to the notion accepted up to now, the hepatic steroid-5β-reductase is an enzyme of low substrate specificity: even after 600-fold purification it was not possible to separate aldosterone-5β-reductase from cortisol-5β-reductase activity of the purified enzyme preparation.[39] However, in contrast to normal plasma cortisol levels in the hypertensives, as demonstrated by the results of our previous studies,[22,25,40] plasma levels of aldosterone were found to be significantly elevated in 45 percent of patients with essential hypertension and normal PRA.*[37,38]

The following deductions can be made from the interrelation of our findings with those of the Genest group:

a) Should increased plasma levels of aldosterone in patients with essential hypertension be the result of the decreased activity of hepatic Δ^4-hydrogenase, the latter must be the initial link in the chain of alterations in the activities of steroid-metabolizing enzymes. If the activity of Δ^4-hydrogenase was not primarily limited, but its decrease was secondary to the increased activity of other aldosterone-metabolizing enzymes (e.g., 18-glucuronyl-transferase or, as in the case of cortisol metabolism, 20-reductase and 6-hydroxylase), there would be no explanation for the increased plasma aldosterone levels unless one postulates an entirely different genesis of this increase. Enhanced aldosterone-binding by plasma protein(s), or an accelerated rate of release of aldosterone from receptor-sites in target tissues come to mind, but for these there is no evidence.

b) Hence, if we assume that the decreased activity of corticosteroid Δ^4-hydrogenase is the initial enzymatic abnormality in patients with essential hypertension, and the increase in the production rates of ring-A nonreduced steroid metabolites is a consequence of it, the latter may or may not completely compensate for the decreased rate of cortisol and aldosterone metabolism through the reduction of the ring-A of the steroid nucleus. Should this compensation be only partial, an elevation of plasma levels of these steroids might ensue. However, while any cortisol excess would promptly result in the suppression of ACTH-release, with a consequent reduction in cortisol secretion rate and the return of plasma cortisol to normal levels, plasma concentrations of aldosterone might stabilize on a higher than normal level, due to the multifactorial and complex mechanism of control of aldosterone secretion.[41] This concept is diagramatically represented in Fig. 2. Moreover, since cortisol-6-hydroxylases and 20-reductases are present also in the liver, that is, within the same metabolic compartment as Δ^4-hydrogenases, these enzymes may act promptly upon the excess of cortisol unmetabolized by Δ^4-hydrogenase, so as not to allow this excess

*In the present study, PRA was not determined in the subjects examined, since at the time nothing was yet known about the existence of "low-renin" and "normal-renin" subgroups of patients with essential hypertension. However, since the "low-renin" subgroup comprises only about 25 percent of all patients with essential hypertension, it would appear from the degree of statistical significance of differences between our normotensives and hypertensives that these differences pertain rather to the larger subgroup, that is to the patients with normal PRA, or to all hypertensives.

to escape into the systemic circulation. In contrast, the major ring-A nonreduced metabolite of aldosterone, 18-glucuronide, is formed predominantly in the kidney.[41] Therefore, in patients with essential hypertension, the excess of aldosterone unmetabolized by Δ^4-hydrogenase is more likely to reach the circulation than that of cortisol.

With regard to the second question, as to whether the detected abnormalities are the cause or effect of elevated blood pressure, certain deductions can be made by interrelating our findings with those of Silah[42] and Turcotte and Silah.[43] These investigators studied activities of the hepatic enzymes cortisol-Δ^4-hydrogenase and cortisol-sulfokinase in rats made experimentally hypertensive by compression of renal parenchyma. The results of those studies revealed a significant increase in the activity of both of these enzymes in the hypertensive rats, this being presumably the result of the elevated perfusion pressure of the liver, subsequent to hypertension. Hence, the decrease in the activity of Δ^4-hydrogenase in patients with essential hypertension, revealed by the results of our study, is unlikely to be the result of hypertension. In contrast, the increase in cortisol-sulfokinase activity, found by us in these patients, is compatible with the findings of Turcotte and Silah, and with the concept that this is a secondary abnormality (either as a sequence of decreased metabolism of cortisol by Δ^4-hydrogenase, or as a result of increased perfusion pressure of the liver,

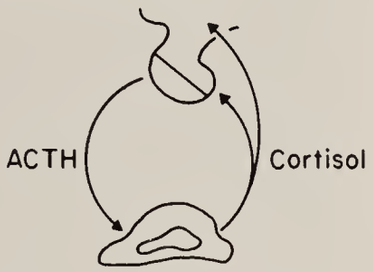
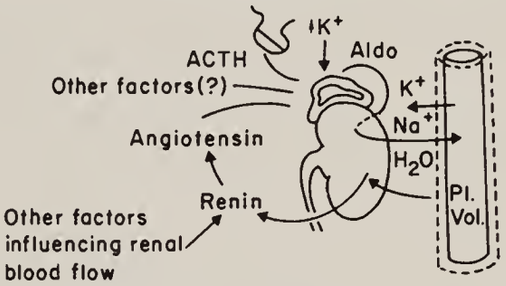
STEROID:	CORTISOL	ALDOSTERONE
Control of Secretion:		
Metabolic Derangement:	(1) Basic: ↓ Δ^4-hydrogenase (2) Sequence: a) ↑ PR of ring-A non-reduced metabolites, compensating for (1) b) if still excess of unmetabolized cortisol present*, ACTH release promptly suppressed	(1) Basic: ↓ Δ^4-hydrogenase (2) Sequence: a) ↑ PR of 18-oxo-conjugate, (and other ring-A non-reduced metabolites), partially compensating for (1) b) multifactorial and complex mechanism of control of secretion incapable of promptly and precisely responding: homeostatic equilibrium reset
Resulting Plasma Levels:	N	↑

Fig. 2—POSTULATED INTERRELATIONSHIPS BETWEEN SECRETION, METABOLISM, AND PLASMA LEVELS OF CORTISOL AND ALDOSTERONE IN PATIENTS WITH ESSENTIAL HYPERTENSION.

*Present only under acute stress; under usual circumstances, there is presumably no excess of unmetabolized cortisol in the liver, and consequently, in the systemic circulation, since cortisol-20-reductases and 6-hydroxylases are present within the same metabolic compartment (hepatic) as Δ^4-hydrogenases (see text).

↑ = increase; ↓ = decrease. PR = production rate. Pl. vol. = effective circulating plasma volume.

or both). Moreover, if we accept that the decrease in Δ^4-hydrogenase activity is the initial link in the chain of the observed enzymatic abnormalities in patients with essential hypertension, it is possible that this decrease may precede hypertension, and be an etiological factor in its development, or may be "associated" with essential hypertension, that is, caused by the same factor, or factors, which lead to hypertension.

It is of interest that several other investigators also found evidence for overproduction of polar corticosteroids with Δ^4-3-keto grouping intact in hypertension: Katz *et al.*,[44] investigated urinary excretion of 6β-hydroxycortisol in normal subjects and in patients with essential hypertension and found it to be higher in the latter, whereas excretion of total 17-OHCS metabolites was within normal range; Touchstone *et al.*,[45] reported that the adrenals of patients with essential hypertension, in tissue incubation experiments, produced significantly larger quantities of 20α- and 20β-dihydrocortisol than the adrenals of normotensive controls; moreover, there was a positive correlation with increase of diastolic blood pressure up to the 120 mm Hg level, and a negative correlation with further increases; Gosh and Pennington[46] found a markedly increased urinary excretion of 6β-OH-20β-dihydrocortisol and its 20α-epimer in pregnancy associated with hypertension, while the excretion of these steroids in pregnant normotensive women was not elevated.

As a working hypothesis it is proposed that the detected abnormalities in the activities of corticosteroid metabolizing enzymes lead to an increase in arteriolar resistance, or to an expansion of plasma volume, or to both, in patients with essential hypertension, this manifesting itself as an elevated arterial blood pressure.

In accordance with such a hypothesis, the causative factors in essential hypertension would be either: (1) increased plasma levels of ring-A *non*reduced steroid metabolites, some of which could be "vaso-active" compounds, thus directly contributing to an increased arteriolar resistance, or (2) increased plasma levels of aldosterone and/or other mineralocorticoids; these could either (a) effect an increase in the circulating plasma volume, through increased sodium and water reabsorption from the renal tubules ("volume hypertension"), or (b) indirectly contribute to increased arteriolar resistance by increasing intracellular sodium ion concentrations in the arteriolar wall, thus elevating the tone of arteriorlar muscles, and, consequently, increasing their responsiveness to other "normally" circulating vasoconstrictive agents ("vasoconstriction hypertension"). The latter stipulation would imply the existence of a mechanism by which an increase in the concentration of intracellular sodium ions could be achieved without a persistent increase in the intravascular sodium pool, and an expansion of plasma volume. A hypothesis to this effect has been recently formulated.[20,47]

Important information as to whether the detected abnormalities in the metabolism of corticosteroids are cause or effect of hypertension would be provided by: (1) conducting a similar study of steroid metabolism in patients with renal hypertension; and (2) determining whether the detected abnormalities in corticosteroid metabolism can be correlated with PRA, that is, whether they occur only in essential hypertensives with normal PRA, or in those with low PRA, or in both subgroups.

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PROSTATE ADENOCARCINOMAS IN WISTAR RATS

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ABSTRACT. An unusual series of spontaneous prostate adenocarcinomas was encountered in germfree Lobund Wistar rats. This report reviews the characteristics of the tumors and assesses their possible applications as experimental models for prostate carcinoma in man, and as a model for the study of metastasis.

Fourteen years ago, there was general agreement among animal biologists that germfree (GF) animals were free of microbial agents and free of spontaneous diseases, other than those due to nutritional deficiencies. Data accumulated since then has clarified important new concepts of endogeneous and of induced diseases in GF animals.

Investigations on GF mice have thus far revealed that all strains carry leukemia virus, several strains carry mammary tumor virus, and one strain carries lymphocytic choriomeningitis virus.[1-4] These viruses are transmitted to progeny via congenital routes; we know of no way to interrupt that transmission route. The GF (gnotobiotic) mice develop diseases related to these viruses, which are similar to the diseases in the conventional strains from which they had been derived by aseptic caesarian section. However, the lesions develop without bacterial complications which in some cases may obscure pathogenic mechanisms. Extensive searches for microbial flora in GF rats have thus far been negative.

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Current diets for GF rats and mice are good enough to prevent nutritional deficiency diseases. In addition, the records of reproduction, growth, and survival of GF rodents compare very favorably with the conventional stock from which they had been derived. Other than the enlarged, thin-walled cecum, they are normal animals. Their lymph nodes and spleens are small and relatively inactive (unstimulated); however, they can be readily activated following exposure to antigen.

As with their conventional counterparts, GF rodents are susceptible to the oncogenic effects of chemical, physical, and viral agents.[5] They develop an interesting spectrum of spontaneous diseases, which, along with those induced experimentally, provide important model systems for neoplastic and immunologic diseases. Also, experimental protocols can be implemented in GF rodents which otherwise would result in bacterial complicacations: *e.g.*, immunosuppressive procedures.

The patterns and types of diseases and of longevity recorded between conventional and GF rats were different.[6-8] Conventional rats developed many diseases and therefore did not live as long as GF counterpart rats: deaths among conventional Wistar strain rats have been attributed to infections, to benign and to malignant neoplasms of endocrine organs and of their target organs. Conventional Wistar rats rarely lived beyond age 20 months. By contrast, up to age 20 months, GF rats rarely developed detectable diseases. Between ages 20 to 30 months, GF

Wistar rats started to manifest increasing numbers of adenomas of endocrine organs and their target organs.[7,8] The source of data acquired from younger disease-free GF rats came from rats which were killed arbitrarily, while fatalities among some of the older rats were associated with large obstructive thymomas or hemorrhagic pituitary glands. Beyond age 30 months, some of their endocrine-related tumors assumed malignant characteristics. The tumor cells appeared immature, many mitotic figures were noted among them, and the cells were found beyond the normal capsular limits of the glands. Transitional malignant characteristics were observed in tumors of breast, pituitary, adrenal medulla, and prostate glands. In addition, several rats developed monocytic leukemia.

Spontaneous prostate tumors have been described in man, dogs, hamsters, and in a Copenhagen rat.[9] Prostate adenocarcinomas occur with highest incidence among men beyond age 50 years. The etiological factors in man are unknown; however, epidemiologic surveys have pointed to higher levels of disease in specific geographically-located human ethnic groups.[10] Herpes virus, of uncertain significance, has been described in human prostate tumor cells by two investigators.[11,12] Lines of human prostate tumor cells have not been propagated *in vitro*. Attempts to induce prostate carcinomas experimentally in animals have failed. Therefore, there is urgent need for an experimental system of prostate carcinomas in animals that reflect the disease in man.

Prostate adenocarcinomas which developed spontaneously among aged GF Wistar rats are the subject of this report.[13] Thus far, we have observed nine prostate adenocarcinomas among aged retired breeders of the 37th generation of GF Lobund Wistar rats (Table I). The rats were born GF and were maintained GF for their lifetime. They were provided sterilized food, water, and bedding. They lived in sterile cages with sterile air. They were examined at frequent intervals for bacteria, and the tests were negative. They had been examined for viral flora with negative results.

Rats which appeared weak, or showed some incoordination of movements or respiratory distress were transferred from the GF system to the autopsy room. Each rat was subjected to complete gross and microscopic examinations for evidence of disease. The prostate glands of eight rats were enlarged and scirrhous, and the kidneys were swollen and greenish in appearance. In several rats, visceral organs had white focal lesions of variable numbers and distribution, which were actually metastatic foci of the prostate carcinomas (Table I). It is likely that the GF status of the rats permitted them to live long enough for the disease to develop.

Prostate tumors from three of the rats were individually excised, minced with scissors in Medium 199, and transplanted subcutaneously (SC) to suckling and to weanling Lobund Wistar rats. Also, tumor fragments were deposited in tissue culture bottles with Eagle's MEM medium plus 10 percent fetal calf serum. It is significant that the three attempts at transplantation were successful, and the transplant series now exceed 20 passages with no change in the morphology of the tumors.[14] Cells from the three tumors have been propagated *in vitro* as monolayers of epithelial cells. Thus far, we have detected no virus in them. Two of the transplanted tumors, arbitrarily designated I and III, were similar microscopically: They had epithelial cells arranged in islands of glandular tissue, separated by considerable connective tissue and fibrinoid material. Tumor I contained numerous myelocytes, while tumor III had few myelocytes. All of the tumors were scirrhous and had varying amounts of central necrotic tissue. The metastatic tumors, predominantly in lymph nodes and lungs, were similar to the primary tumors. Following subcutaneous implantation of tumor cells into the dorso-lumbar region, a nodule appeared within two weeks which enlarged and ulcerated through the skin with the development of a black eschar. Within days, distinct expanding tumor

TABLE I

ADENOCARCINOMAS OF THE PROSTATE GLANDS IN GERMFREE LOBUND WISTAR RATS

Rat No.	Age/ Months	Body Wgt./Gms	White Blood Cells/Mm³	Hema-tocrit %	Spleen Wgt./ Gms	Prostate Tumor Wgt./Gms	Tumor Metastases
1	22	302	15,700	27	0.31	N.D.	Lungs
2	32	484	26,000	38	1.5	12.0	None
3*	37	378	—	—	0.75	7.0	Multiple organs**
4	39	385	71,000	38	1.4	34.0	Multiple organs**
5	35	465	8,400	42	1.09	6.0	Multiple organs**
6	37	388	4,000	27	1.0	20.8	Multiple organs**
7	31	381	26,000	24	1.73	13.2	Multiple organs**
8	38	424	4,200	48	0.8	N.D.	Lungs
9	38	629	11,800	34	1.4	N.D.	None

*Dead on arrival in laboratory.
**Spleen, liver, peritoneum, lymph nodes, lungs.

foci were detected in the ipsilateral axillary lymph node and in and on the lungs. Tumors I and III have been propagated *in vitro* as monolayers of epithelial cells which secreted acid phosphatase, and as few as 10 cells induced characteristic metastasizing adenocarcinomas in GF and in conventional Wistar rats. Wistar strain rats from two other sources rejected these tumor cells.

Prostate tumor II differed histologically from the other two transplanted tumors: It was made up of packets of large epithelial cells with little connective tissue cells. Following subcutaneous implantation, the tumor grew to very large masses which rarely penetrated the skin. The tumor cells spread to lymph nodes, and to the lungs. Lung lesions of prostate II tumor were more diffuse than the solid focal lesions developing from tumors I and III.

In the search for experimental models of prostate carcinomas, we had to define the rat tumors as prostate in origin, and then to explore their possible applications to the problem of prostate cancer.

Eight of the autochthonous tumors were similar morphologically, they were derived from markedly enlarged prostate glands, and metastatic foci thereof were unchanged. In two of the rats, prostate carcinomas were still surrounded by normal prostate tissue.

We have applied this experimental tumor system to (a) the phenomenon of metastasis, to (b) therapeutic effects of immunostimulants, to (c) cytotoxic cyclophosphamide, to (d) estrogen analogues, and to (e) the modifying effects of allogenic bone marrow chimerism in irradiated tumor-bearing GF rats.

Some practical application of this tumor system in Wistar rats may be of interest: The results were assessed by tumor weight and by metastatic lesions in the lungs. Within 10 days after subcutaneous inoculation of 10^5 prostate tumor I cells, minute tumors appeared in the cortical areas of the ipsilateral draining axillary lymph node; and thereafter in and on the lungs. Distinct round tumor foci could be enumerated visually at 30 days by inflating the lungs through the trachea with Bouin's solution, and 24 hours later storing them in 70 percent ethanol. Defined metastatic lung tumors appeared white against a yellow background.

(A) Immunostimulation. Simultaneous intravenous inoculation of killed Corynebacterium parvum (2.1 mg) and tumor cells, modified inconsistently the metastatic spread of tumor cells to lymph nodes and lungs (Table II). Growth of the subcutaneously implanted tumor cells was not affected.

(B) Cyclophosphamide (5 mg) was injected into GF rats intraperitoneally (IP) at weekly intervals. When administered at week one after inoculation of tumor cells, tumors failed to grow, or were very small at the implant site; and lesions in

lymph nodes and lungs were absent (Table III). When treated at day 30 following inoculation of tumor cells, subcutaneous tumors were relatively small and the numbers of lung metastases were reduced and abnormal in appearance (Table IV).

(C) Diethylstilbestrol (0.05 mg) in oil was administered intramuscularly (IM) at daily or at semi-weekly intervals to rats with SC implanted prostate tumor I cells. The effects on the tumor growth pattern was inconsistent: In many cases the SC tumor was not changed, but the numbers of metastatic lesions were reduced. The drug was toxic as reflected by failure of the animals to gain weight (Table V).

It is noteworthy that application of the same diethylstilbestrol treatment protocol to rats with prostate tumor II resulted in enhanced growth of the tumor (Table VI).

(D) In earlier experiments, lethally irradiated GF Wistar rats which were reconstituted with Sprague-Dawley bone marrow cells survived indefinitely. Conventional counterpart rats subjected to the same experimental protocol died of so-called graft-versus-host disease. GF Wistar rats with detectable subcutaneous prostate tumors were given whole body irradiation (900 R). Twenty-four hours later, each was inoculated I.V. with the equivalent of two femur contents from GF Sprague-Dawley or Wistar rats. They survived without lung tumors for three weeks thereafter, while untreated control rats developed extensive lung metastases (Table VII).

TABLE II

PROSTATE CARCINOMA I—TREATED WITH CORYNEBACTERIUM PARVUM*

	Weights			
	Spleen	Liver	Tumor	Lung Tumors
C.P. control	0.67	10.25		
Tumor control	0.70	9.31	0.69	30
Tumor & C.P.—4 d.	1.13	9.93	1.39	78
Tumor & C.P.—0 d.	0.89	10.41	1.43	15
Tumor control	0.61	7.0	2.2	10.4
Tumor & C.P.—0 d.	0.98	10.3	1.7	0
Tumor & C.P.—4 d.	0.89	6.83	2.1	0

*0.3 ml I.V. (Wellcome)

TABLE III

GERMFREE PROSTATE CARCINOMA—TREATMENT WITH CYCLOPHOSPHAMIDE*

No.	Sex	Treatment	Body Wt./Gm	Tumor Wt./Gm	Spleen	Lung Metastases
1	F	Control	162	2.40	0.52	204
2	M	Control	250	6.35	—	671
3	M	Control	256	7.47	—	386
4	M	Control	244	15.00	0.84	619
1	M	CPA*	268	None	0.53	None
2	F	CPA*	158	0.08**	0.26	None
3	F	CPA*	158	0.15**	0.22	None
4	F	CPA*	163	0.14**	0.23	None
5	F	CPA*	170	0.25**	0.24	None
6	F	CPA*	152	None	0.25	None
7	F	CPA*	132	0.27**	0.28	None
8	F	CPA*	158	0.03**	0.24	None

*5 mg Cyclophosphamide (CPA) I.P./week/6 weeks. Started at one week after S.C. inoculation of tumor cells and killed for examination at one week after last dose of CPA.
**Scar tissue with occasional intact tumor cell.

TABLE IV

GERMFREE PROSTATE CARCINOMA—TREATMENT WITH CYCLOPHOSPHAMIDE*

No.	Sex	Treatment	WBC/Mm³	Hematocrit %	Weights/Gm				Lung Metastases
					Body	Tumor	Spleen	Liver	
1	M	Control	14,100	54	256	5.72	0.78	7.64	385
2	M	Control	17,500	44	170	6.1	0.73	6.6	305
1	F	CPA	3,900	41	157	0.74	0.25	5.25	11
2	M	CPA	6,100	47	310	1.5	0.65	11.1	19

*5 mg Cyclophosphamide (CPA) I.P./week. Started one month after implantation, for 4 weeks, and killed one week after last treatment. Control rat, after one month, had 50 lesions in lungs.

TABLE V

PROSTATE CARCINOMA I—DIETHYSTILBESTROL TREATMENT*

	No. and Sex	Wt. (Range)	Tumor Wt. (Range)	Gonads	Lung Metastases (Range)
DES	4F	130 (109-160)	2.9 (2.2-4.0)		54 (24-92)
	3M	165 (147-184)	3.4 (2.2-4.9)	0.2	60 (39-120)
Controls	3F	156 (152-159)	4.6 (3.9-5.5)		152 (64-225)
	2M	271 (261-282)	5.4 (4.2-6.7)	1.1	190 (179-201)

*0.5 mg DES I.M. daily—starting one day after transplantation.

TABLE VI

RATS WITH PROSTATE CARCINOMA II—TREATED WITH DIETHYLSTILBESTROL*

Examination At		No. Rats	DES Schedule	Wt.	Tumor Wt. (Range) Mg.	Gonads Wt. Mg.
5 weeks	Controls	3	—	340	2.54 (1.08-3.7)	1.2
	Treated	5	Daily	296	11.2 (1.6-27.5)	0.53
	Treated	6	Daily	278	30.5 (2.8-41.00)	0.19
	Treated	6	2X/wk.	344	12.94 (3.0-28.2)	0.96
8 weeks	Controls	3	—	384	21.69 (11.3-33.9)	1.25
	Treated	6	2X/wk.	344	42.64 (0.1-81)	0.33

*0.5 mg I.M.

TABLE VII

EFFECTS OF SYNGENEIC AND ALLOGENEIC BONE MARROW CHIMERISM ON TRANSPLANTED PROSTATE CARCINOMA I IN WISTAR RATS*

Bone Marrow Transplanted	Rats	Tumor Wgt./Gm	Lung Metastases
A. Sprague-Dawley	6	1.30	91
Wistar	2	1.67	63
Untreated controls	4	2.68	447
B. Sprague-Dawley	4	0.65	4
Untreated controls	4	1.58	39
C. Sprague-Dawley	4	0.76	70
Wistar	4	0.97	33
Untreated controls	4	1.05	84
D. Sprague-Dawley	4	1.86	197
Wistar	4	3.20	312
Untreated controls	4	8.25	702

*Each rat was administered 900 R x-rays (whole body), and 24 hours later inoculated I.V. with bone marrow cells from 2 femurs. The rats were killed 2 weeks later, and examined for gross and microscopic evidence of disease.

Experimental pathologists are searching for models of disease which simulate counterparts in man. This report describes prostate tumors which appeared spontaneously in aged Wistar rats. The rats had metastatic tumors. Transplanted tumor cells maintained their characteristic disease pattern, which makes them useful models for studies on tumor growth and metastasis. There is much to be done with the prostate tumor system described here. We are providing these tumors to other investigators with the hope that they will contribute to our better understanding of carcinoma of the prostate gland.

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THE POSSIBLE ROLE OF SERUM AND URINARY MYOGLOBIN DETERMINATIONS IN ASSESSMENT OF MYOCARDIAL INFARCTION: CLINICAL PERSPECTIVE

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ABSTRACT. Myoglobinuria has been detected in a large percentage of patients with acute myocardial infarction. The usefulness of urinary and plasma myoglobin analyses might be of considerable importance in qauntification of infarct size. The development of such techniques should include evidence for: (1) specificity of myoglobin determinations; (2) establishment of correlation between infarct size and total plasma or urine myoglobin; (3) determination of cardiac myoglobin kinetics; and (4) assessment of possible differences between skeletal muscle and cardiac myoglobin which could alter differentiation.

The development of such a method could supplement the use of serial creatine phosphokinase (CPK) determination in studying myocardial infarction.

The quest for rapid quantitative assessment of the volume of infarcted myocardium has led to the use of serial creatine phosphokinase analyses and precordial multi-lead mapping. The search for a readily analyzable specific product of recently infarcted cardiac muscle continues.

As early as 1940, Prinzmetal[1] suggested that myoglobinuria might be the one specific diagnostic feature of myocardial infarction. Several recent studies[2-5] have indicated the presence of myoglobinuria in a large percentage of patients with early myocardial infarction. These studies have involved immunochemical determinations, since the amount of myoglobin released into the urine under conditions of infarction appears to be small, in contrast to the myoglobinuria of skeletal muscle disease or injury.

Study of the potential efficacy of the use of myoglobin determinations in serum and plasma to quantify myocardial damage should involve assessment of (1) specificity of present determinations in quantifying urine and plasma myoglobin; (2) specificity of myoglobin levels in relation to infarction; (3) determination of the kinetics of myoglobin; (4) establishment of possible differences between skeletal muscle and cardiac myoglobin which could allow differentiation.

QUANTIFICATION OF MYOGLOBIN IN URINE AND SERUM

Recent immunochemical methods have allowed quantification of small amounts of myoglobin in serum and plasma.[6-10] These methods include immunodiffusion and microflocculation.

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A recent abstract by Lwebuga-Mukasa et al,[11] suggests that serum radioimmunoassay may be the most sensitive determinant of myoglobin. Using ^{125}I myoglobin, the serum levels of myoglobin of six dogs with experimental myocardial infarction were serially studied. Myoglobin appeared in the serum within two hours of coronary occlusion and reached a peak level in six hours, disappearing within 12 hours. Serum levels were in the nanogram range, the peak averaging 250 ng/ml.

Improved immunologic techniques using adjuvants allowed the use of prepared antibodies to myoglobin in the early 1950's and this extended to clinical evaluation a decade later. Kagen,[12] using radial diffusion to agar pre-mixed with specific human antiserum, could determine levels as low as 15 μg/ml, and with double diffusion gel technique, levels between 5 and 15 μg/ml using immunodiffusion, and 0.04 μg/ml using microflocculation. Sources of error in immunodiffusion may be due to large amounts of albumin which may remove the heme group from myoglobin, to form methemalbumin.[13] The urine, while giving just as strong a peroxidase reaction as originally, will no longer react with antibody. Highly alkaline urines may also interfere with hemagglutination-inhibition tests.

The most sensitive method of detection of myoglobinuria by immunologic techniques has been hemagglutination-inhibition. Antiserum dilution may allow sensitivity of 0.3 μg/ml, should hemoglobin be absent. Immunodiffusion techniques are much less sensitive.[13] The normal levels of myoglobin in the urine have not been defined. There is normally no evidence of myoglobin in either urine or plasma using hemagglutination-inhibition techniques. With vigorous muscular activity, myoglobin in urine sometimes increases to detectable levels.[13] Although these techniques can detect small quantities of myoglobin, the ability to measure precisely these amounts has yet to be determined. Interfering factors mentioned previously may affect values even with a reasonably accurate quantifying technique.

SPECIFICITY OF MYOGLOBIN CHANGES IN ACUTE INFARCTION

Most studies have focused on semiquantitative myoglobin determinations in urine of patients during acute infarction. Antiserum to human or monkey myoglobin has been used for immunologic determinations. Adams and Elliott[3] found myoglobinuria in 35 of 44 patients with acute infarction, and in 16 of 36 with cardiopulmonary disease without infarction. Levine et al.,[2] using a hemagglutination-inhibition test, found myoglobinuria in 89 percent of patients with definite infarction during the first day of hospitalization, and in over 75 percent during the first three days of hospitalization. A false-positive test was noted in only one of eight patients without infarction. Bernstein et al,[5] using a precipitin reaction, found levels of over 500 μg/ml in urine in 49 of 50 patients with acute infarction during the acute period. No evidence of myoglobinuria was found in 50 patients with coronary insufficiency without infarction, and in 20 normal subjects.

Most recently, we have studied 23 patients with evidence of acute infarction, using the time of the acute event as a factor in determination of positivity of urine myoglobin.[4] Of 11 patients evaluated by hemagglutination-inhibition techniques during the first 24 hours of symptomatology, nine had positive urinary myoglobin. In all, 15 of 22 patients with definite infarction had myoglobinuria. Of 15 patients with possible infarction but no definite electrocardiographic or enzyme evidence, none had myoglobinuria.

Thus, recent studies suggest that myoglobinuria may be present in a great percentage of patients with infarction during the first 24 hours, and that there are only rare false-positive reactions. The simplicity and rapidity of the test using hemagglutination-inhibition suggests that this test may be a useful rapid screening procedure.

There have been no reported studies of attempts to quantify changes in urine and serum levels serially.

PHARMACOKINETICS OF MYOGLOBIN

Myoglobin may be released into the plasma as a result of damage to skeletal or cardiac muscle. It has a molecular weight of 17,000, and does not bind to haptoglobin. Thus, it is rapidly excreted by the kidney. The actual renal threshold of myoglobin is not known. Bywaters has found myoglobinuria in plasma levels below 3 μg/ml.[14] Myoglobin might be metabolized as well by the reticulo-endothelial system, or destroyed in the urine by myoglobinolytic enzymes active in urine at acid pH.[12,15,16] In a study of serum and urine myoglobin in myositis, Kagen[12] found myoglobin in the serum of 68 percent of patients, and in urine in only 20 percent. Thus, although the threshold may be low, sensitive immunologic techniques may still fail to detect myoglobinuria in the presence of increased serum myoglobin.

Most studies of myoglobin have involved skeletal muscle disease or injury, which may cause prolonged excretion and high serum levels of myoglobin for prolonged periods. Myocardial infarction may be a better model for myoglobin kinetics, since necrosis occurs rapidly, and presumably, myoglobin from necrotic myocardium would be released over a short period of time, if we can draw a parallel with serum CPK activity after infarction.

Study of myoglobin kinetics under these circumstances should be pursued in animal models of myocardial infarction, where serial serum and urine myoglobin levels can be evaluated in conjunction with serial CPK and electrocardiographic precordial mapping. The development of mathematical definitions of myoglobin disappearance and relation of total serum myoglobin to volume of necrosis of myocardium should be studied. Of importance would be a relation between urine and serum myoglobin levels under these circumstances. This would constitute a truly non-invasive evaluation of infarction size.

ISOLATION OF A SPECIFIC MYOGLOBIN FROM MYOCARDIUM

The final step in quantification of infarct size would be the definition of a specific myocardial myoglobin. There is no difference in electrophoretic mobility or peptide chromatograms of human skeletal and cardiac myoglobin.[17] It is unlikely that radioimmunoassay or immunochemical methods would be able to distinguish the two, and only subtle differences in peptide sequence may be present. It therefore seems unlikely that there would be a specific cardiac myoglobin. However, the infrequency of appearance of myoglobinuria or myoglobinemia, except in obvious skeletal muscle disease, suggests that myoglobin elevation *per se* in acute infarction may still be an accurate means of demonstrating infarct size, should serial quantification be readily available.

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RESEARCH INTO THE CAUSES AND THERAPY OF INFECTIOUS DISEASES IN PRE-WORLD I RUSSIA

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EDITOR's NOTE: This is the fourth in Dr. Bezkorovainy's series of articles on the history of medicine and allied sciences in Russia.

ABSTRACT. Because infectious diseases were quite prevalent in Russia during the pre-World War I era, a number of Russian physicians and microbiologists dedicated themselves to the study of infectious agents. The most prominent among the cholera and plague researchers were Zlatogorov, Zabolotny, Isaiev, and the expatriate Haffkine.* Spirochetal disorders were successfully studied by Motchuthowsky, Vasiliev, and Gabrichevsky. Sakharov was first to establish the presence of spirochetes in animal blood. Parasitic diseases were also well studied by the Russians, the most accomplished of whom were Romanovsky, the malaria researcher and discoverer of the so-called Romanovsky stain; and Borovsky, who discovered the causative agent of oriental sore.

INTRODUCTION

In the past one hundred years we have witnessed a dramatic rise in the human life expectancy, and this is ascribed principally to the conquest of various infectious diseases such as smallpox, cholera, tuberculosis, typhoid fever and the various childhood diseases. In the European continent of the 19th and early 20th centuries, infectious diseases were most prevalent in Russia, chiefly because of its proximity to the Asiatic endemic centers for cholera and plague, its large rural population with a relatively undeveloped industrial base, and the scarcity of medical facilities therein.[1] Under the circumstances, one would expect that research on the nature of the causative agents of infectious disease should have been one of the priority targets in Russian medical schools and scientific institutes of the pre-World War I era. Indeed, microbiology was one of the more popular areas in which many Russian researchers concentrated. Though they did not succeed in discovering the causative agents of any of the major infectious diseases, they, nevertheless, made significant contributions to the understanding and methods of treatment of these diseases.

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*Proper names of Russian origin have to be romanized phonetically from Cyrillic orthography. The spelling as published from time to time may vary, depending on the European language involved. For instance, we are accustomed to see "Romanowsky" and "Bechterew" because German literature established the precedent; likewise, "Haffkine," rather than "Chavkin" and "Metchnikoff" for "Mechnikov" because of French associations and phonetics. *Ed.*

CHOLERA AND PLAGUE

Cholera made its first appearance in Russia in 1823. In that epidemic alone, which originated in India, approximately 200,000 persons died.[2] The disease made frequent reappearances in Russia until the 1930's. Plague was much less frequently encountered in Russia,[3] though there were areas in the eastern part of the country

that experienced the periodic flareups of this disease. Plague too was not wiped out until the 1930's.[4]

Among the early physicians who did considerable work on the etiology of the cholera was Carl Rosenberger (1805-1866), a native or Dorpat and a naval physician.[5] He is credited with having singlehandedly put down in 1830 a mutiny on board a vessel of the Black Sea flotilla, where a plague epidemic had broken out. In 1836 he left the navy, and joined government service in a civilian capacity. As such he directed the first successful battle against a cholera epidemic in Russia, emphasizing preventive measures to contain the disease.

Another notable cholera expert was Leonty Rklitsky (1815-1867),[5] a professor of the Military-Medical Academy in St. Petersburg. In 1843, he published in Russia's "Military-Medical Journal" a method of treating cholera patients with salts. It is today well known that cholera causes a dramatic loss of body electrolytes, and successful treatment must include an intravenous replenishment of these electrolytes.

The most famous cholera and plague fighter of Russian origin was, of course, Waldemar Haffkine (Vladimir Aronovitch Chavkin).[6] He was born in 1860, in Odessa, in the family of a Jewish merchant, and graduated from Odessa University in 1883 with a degree in zoology. He then worked in a laboratory of the Odessa Zoological Museum, and in 1888 left Russia for Geneva, Switzerland. A year later he joined the Pasteur Institute in Paris, where he worked on the etiology of infectious disease. In 1892 he was able to report the production of a cholera vaccine, and petitioned the Russian Government for permission to test it in Russia. His request was denied, however, apparently because of the sad results of Ferran's experiments with "variolization" against cholera in 1885.[7] Instead, Haffkine was invited to test his vaccine in Calcutta, India, where he was able to reduce mortality from cholera by 72 percent in vaccinated populations.

Haffkine's anti-cholera vaccine was prepared according to the principles enumerated by Jenner, and was administered in the form of two subcutaneous injections. The first injection consisted of attenuated organisms, i.e., organisms grown at 39° under aeration with sterile air. The second injection alone did not provide a significant immunity against cholera, nor did both injections when given in small doses. However, both injections given in maximal quantity resulted in 17-fold lower mortality and 19-fold lower morbidity rates from the disease in vaccinated populations. Protective effects of the vaccine lasted from the 11th day following vaccination until at least the 450th day.[7] The Haffkine-type cholera vaccine was regularly used throughout the world, and only recently a more effective vaccine, based on the immunization against the vibrio toxin, has been described.[8]

In 1896 Haffkine moved to Bombay to see what could be done to halt the plague epidemic raging in that area. Using the newly discovered Bacillus pestis (Yersin and Kitasato in 1894), he was able to prepare an effective plague vaccine, which served to decrease mortality by 80 to 90 percent in vaccinated populations. During an inoculation program in Malkowal, a few of the vaccinated villagers died from tetanus, the microorganisms of which had apparently contaminated one of the vaccine bottles. Haffkine was severely attacked by his critics for this so-called "Malkoval disaster," and it took him some time to exonerate himself. It is unfortunate that such an accident would elicit so much abuse upon a man who had done so much for that area of the world. It also may be mentioned that Haffkine's efforts were not always enthusiastically supported by local Indian authorities. He was even periodically accused of being a Russian spy.

In preparing his plague vaccine, Haffkine attempted to make a preparation that would provide the patient with dual protection against the invasion by the microorganism and against its toxins.[9] However, he apparently settled for a vaccine against

the invasion by the bacillus only. Haffkine grew the plague bacilli suspended from a layer of butter or oil on top of a nutrient broth. The bacilli grew down into the broth in the form of "stalaktites" and were periodically shaken to collect at the bottom of the flask. After a six-week growth period, the bacteria were collected and inactivated by heating to 65 to 70°. The vaccine was then tested in several Indian jails, then in larger populations in the Portuguese colony of Damoan and the domains of the Aga Khan. Mortality was reduced by 80 to 90 percent in the inoculated populations.

Haffkine retired in 1914 and settled in Paris. He dedicated his remaining years to religious study and the promotion of cultural awareness among Jewish youth. In 1928 he moved to Switzerland, where he died in 1930.

Haffkine was a zoologist, turned bacteriologist, turned public health physician. A man of his caliber and interests could have been extremely valuable to his mother country with its chronic cholera and plague problems. Yet one wonders whether or not Haffkine could have contributed so much to the world had he remained in Russia, even in the capacity of a university professor, as was his desire. At the Pasteur Institute, Haffkine was, so to speak, at the right place at the right time to become interested in the potentialities of immunization, and it is doubtful that he would have received this stimulation anywhere in Russia.

Cholera vaccination programs in Russia were wide-spread, though not sufficiently so as to totally control the disease. The 1906-1908 epidemic killed 60,000 persons. One such vaccine was prepared by Korwacki from the Warsaw Department of Health,[10] who treated the vibrios first with heat at 56° for one hour, and then with 0.5 percent phenol. The vaccine was administered in three doses at five-day intervals, and the sera of the vaccinated persons showed vibrio agglutinating activity at dilutions of 1:200 to 1:400, whereas convalescents had a titer of 1:200. Korwacki also noted that the standard Russian "Kharkov" vaccine gave similar results. Zabolotny also mentioned that vaccination was administered *en masse* in St. Petersburg during the 1907-1908 epidemic. Among a group of 30,000 vaccinated individuals, only 12 contracted cholera, and four of them died. The incidence of the disease among the non-vaccinated populace, on the other hand, was 68 per 10,000, with about 50 percent of mortality.[11] These results were even better than those obtained by Haffkine in India.

The possibility of passive immunization against cholera was investigated by Fedorov from the Pathological Anatomical Institute in Moscow.[12] He was able to protect rabbits against cholera by injecting them with the serum of immunized rabbits. The animals were protected against the disease even if administered during the incubation period.

The nature of immunity against cholera in immunized animals was investigated by Isaiev (1854-1911) in cooperation with Pfeiffer. Isaiev was a naval physician, first with the acting fleet, then as the chief physician of the naval hospital at Kronstadt. His research work was done during his sojourn with the Institute for Infectious Diseases in Berlin. Isaiev was able to produce temporary immunity to cholera in guinea pigs by injecting into the peritoneal cavity various substances such as serum from healthy human beings, bacterial growth media, urine, and even physiological saline. He showed that the irritation produced in this fashion attracted macrophages to the locus of irritation resulting in a four-to-five day immunity to a challenging dose of virulent cholera bacilli.[13] In the same paper, Isaiev reported on the active and passive immunization of guinea pigs against cholera. Vaccinated guinea pigs were resistant against the disease; however, this resistance was directed against the whole organism rather than against the bacterial toxin. Serums from convalescent human cholera patients also protected the guinea pigs against the disease, and the immunity lasted much longer than that produced by the non-specific irritants listed

above. Such immunity was not entirely due to the presence of macrophages, according to Isaiev, though he failed to speculate on the nature of such secondary immunizing factors.

The mechanism of inactivation of the cholera bacilli by immunized guinea pigs was elucidated by Pfeiffer and Isaiev.[14] They observed that when vibrios were injected into the peritoneal cavity of the animals, the bacteria were immediately inactivated by lysis. The peritoneal cavities became sterile within a very short period of time. They, moreover, observed that the macrophages and other white blood cells did not take part in the immune reaction, and concluded that the immunity was carried out by an as-yet-unknown serum factor. Soviet sources indicate that bacteriolysis was discovered by Isaiev independently of Pfeiffer. This appears not to be true, since both investigators worked on the problem together. However, the lysis of bacteria by immune sera is often referred to as *Pfeiffer's phenomenon* in western literature. In all fairness, the process should be referred to as the *Pfeiffer-Isaiev* phenomenon.

The most accomplished cholera researcher, whose activities were confined to Russia, was probably Simeon I. Zlatogorov (Goldberg?) (1873-1931). He was born in Berlin, but went back to Russia to study medicine at the Military-Medical Academy in St. Petersburg, graduating in 1897. He was then variously associated with the Military-Medical Academy, the Women's Medical College of St. Petersburg, and Zabolotny's laboratory at the Institute of Experimental Medicine. From 1911 to 1917 he worked in Bekhterev's Institute, and during the Soviet era, he was once again associated with the Military-Medical Academy and later with Kharkov University. Soviet sources have accused Zlatogorov of spreading the "reactionary" Mendelian genetic theory with respect to the reproduction of microorganisms.

Zlatogorov became interested in the etiology of cholera as a result of the devastating cholera epidemic of 1906-1908. He travelled to the most heavily stricken area of Russia, the Volga basin, and investigated the mode of transmission of the disease. Taking 89 water samples from the Volga river in the vicinity of Saratov, Zlatogorov found vibrios in 19 of them. He also noted that no sewage-treatment facilities existed in Saratov and Tsaritsin (later Stalingrad, now Volgograd), the raw sewage being first collected in special ravines and then pumped into the Volga river. In addition, the drinking water, taken from the Volga river, was in some instances treated very inadequately ("American filters"), and vibrios were found in such water samples. The filtered water ("British filters"), on the other hand, contained no vibrios. Zlatogorov concluded that contamination of the water supply was the main mode of cholera transmission, and his conclusions were substantiated by the fact that the epidemic was confined to those areas which obtained their water from the Volga river. Cholera cases further inland were rare. Zlatogorov further wrote that such epidemics could be controlled only when the city and other local governments were willing to appropriate funds for the construction of adequate waterworks and sewage treatment plants, and this could come about only through the democratization of the local power structures.[15]

Having returned to his laboratory in St. Petersburg, Zlatogorov proceeded to investigate some of the fundamental properties of the cholera vibrio. He noted that some of the cultures he had brought back had lost their power to agglutinate with the cholera antiserum. Investigating this further, he found that the vibrios could assume a saprophytic character in water, and would be neither virulent, nor would agglutingate with the specific antisera. These cultures could, however, be transformed into the virulent variety by either *in vivo* or *in vitro* culturing and transfers. On the other hand, he could transform the virulent vibrios into the non-virulent type by permitting them to stand for seven days in water at room temperature followed by repeated washing with water.

He concluded that the lack of agglutination does not necessarily indicate the absence of cholera microorganisms.[16]

The duration of the carrier state in convalescing cholera patients was also investigated by Zlatogorov.[17] Such former patients were found to be carriers of vibrios that were not agglutinable with vibrio-specific antiserum, but like the vibrios inactivated in water, could be restored to their virulent state by a variety of *in vitro* methods.

Zlatogorov's contributions were not limited to cholera, and he is credited with providing the first comprehensive description of the properties of the bubonic plague bacillus *(Bacillus pestis* or *Pasteurella pestis)* after its discovery by Yersin and Kitasato in 1894. Zlatogorov investigated 22 different *B. pestis* cultures obtained from either animals or human beings from different parts of the world, including Russia, South Africa, India, South America, France, England, and Mongolia.[18] No significant differences among these cultures were observed. All were gram-negative and indole negative, and all were able to form capsules. All had bipolar staining properties. The bacilli were found to exist in several forms, the most common of which was oval-shaped 0.8-1.5 μ in length and 0.5-0.8 μ in width. They were not motile. The optimum growth temperature was 30°, and the bacilli grew best in the presence of 0.5 percent NaCl and in a slightly alkaline medium. The best growth medium proved to be calf meat broth. Zlatogorov noted the characteristic "stalaktite" growth pattern of the bacilli, which had been observed previously by Haffkine, and observed that the bacilli were also arranged into long chains. He called them "streptobacilli" for this reason. In older cultures the "stalaktite" growth pattern was seen to give way to growth at the bottom of the flask with a thin layer of bacteria remaining on the surface of the medium as an oily layer. Biochemically, the *B. pestis* cultures did not liquefy gelatin, did not convert fructose and lactose to alcohol, and produced alkali during the growth period. The bacilli did not form spores and were completely inactivated at 60°. The growth was much more rapid on the semi-solid agar, whereby a tough membrane was formed over the agar surface. Zlatogorov noted that the pseudotuberculosis bacillus *(B. pseudotuberculosis rodentium Pf.)* was in most respects identical to the plague bacillus. This included all the staining and biochemical properties, and even the ability to agglutinate with sera from patients having recovered from the plague. The only difference was that the antiserum to the plague bacilli was able to form a precipitate with the *B. pestis* culture filtrates (toxin-antitoxin precipitin?), whereas no such precipitate was formed with the filtrate of the pseudotuberculosis organisms.

SPIROCHETAL AND RELATED DISEASES

Zlatogorov's interest in infectious diseases was undoubtedly stimulated by Daniil K. Zabolotny, the director of the laboratory of St. Petersburg Institute of Experimental Medicine in which Zlatogorov worked. Zabolotny (1886-1929) was an active investigator of cholera, plague, syphilis, and a number of other infectious diseases, for which purpose he participated in expeditions to India, Manchuria, and Arabia. He was a graduate of the Odessa and Kiev Universities, and in 1898 became simultaneously professor of bacteriology at St. Petersburg University, the St. Petersburg Women's Medical College, and the director of the syphilis laboratory of the Institute of Experimental Medicine.

While still a student, Zabolotny is said to have prepared, under the supervision of Savchenko, an anti-cholera vaccine, which he used to immunize himself (1893). He then apparently proceeded to inject himself with live vibrios without contracting the disease. His expeditions to Asia were mostly concerned with the study of endemic loci of the plague. During that time he demonstrated that bubonic and pulmonary plague were caused by the same microorganism in monkeys.

D.K. Zabolotny, cholera and relapsing fever researcher.

He also showed in 1910-11 that the persistence of plague in certain areas of Asiatic Russia was due to small wild rodents that were the reservoirs of the disease.

Syphilis was produced in baboons by Zabolotny independently of Mechnikov in 1903, though Zabolotny published his results a year later than Mechnikov in the "Russian Journal of Skin and Venereal Diseases" (1904), and did not receive the publicity his competitor received. Soviet sources make the claim that Zabolotny had observed the spirochete in syphilitic patients some two years before Schaudinn and Hoffmann announced their discovery in 1905. However, in describing the agglutination of spirochetes by serum obtained from syphilitic patients, Zabolotny never made any claim to the discovery of this microorganism.[19]

The presence of spirochetes in the blood of animals was first demonstrated by M. N. Sakharov of Tiflis in the Caucasus, who was in the employ of the Russian railroad system.[20] During his studies of the etiology of malaria, he noted that every summer there appeared a disease among the geese near certain railroad stations, which would cause them to lose weight, cause diarrhea, and would eventually kill all of them. No microorganisms had been identified in the blood and organs of the deceased animals; however, Sakharov was able to show the presence of spirochetes in the blood of the living animals up to one day before death. He was able to transfer the disease from one goose to another, but not from geese to other birds. The spirochetes were very fragile, and were easily crushed between the microscope slide and the coverglass if care was not taken to prevent this. Sakharov was not able to culture the spirochete *in vitro*. Since the microorganism attacked geese only, Sakharov named it *Spirochaeta anserina*.

Another spirochetal disease that assumed major importance in Russia was recurrent fever *(Febris recurrens)*. It made its appearance in Moscow and St. Petersburg in 1864-65, and possibly earlier in the more remote areas of Russia. The clinical progress of the disease was described by several St. Petersburg and Moscow physicians of that time, most notably by Zorn,[21] who proposed that the classical and biliary forms of recurrent fever were one and the same disease, and by Botkin,[22] who, on the basis of certain similarities between malaria and recurrent fever, treated his patients with quinine. The most accomplished investigator of the recurrent fever in Russia was probably Osip O. Motchutkovsky (1845-1903), who was a graduate of Kiev University (1869), a staff physician of the Odessa Municipal Hospital (to 1893), and finally a professor in the Post-graduate Medical Institute in St. Petersburg. Using human volunteers, Motchutkovsky was able to transmit recurrent fever from ill to healthy individuals by injections of infected blood.[23] He thus proved that insects could be carriers of the disease. He could not transmit typhoid fever by this means, nor could he infect the animals with the human blood. Blood from the patients un-

dergoing the acute phase of the disease (pyrexia) only was infective, whereas apyrectic blood from the same patients was not.

Motchutkovsky also investigated various means for predicting the recurrence of acute phases in patients with recurrent fever, and found the previous temperature chart of the patient to be most helpful in this regard:[24] if, during the remission period, the patient's temperature rose steadily, a relapse occurred in 90 percent of the cases, whereas if temperature remained stable no relapse could be expected.

There were apparently no effective therapeutic measures available once the disease had set in. Gabrichevsky (1860-1907) was probably one of the first who made an attempt to prepare a vaccine against the disease by taking blood from a patient during his febrile episode, heating the blood to 56°, and reinjecting it into the patient during the apyretic period.[25] The experiment was not successful. It may be noted that at that time (i.e., 1905) no one had succeeded in culturing the spirochete of relapsing fever *in vitro*. Gabrichevsky believed that spirochetal infections in man, apes, and geese gave rise to humoral antibodies that appeared to be both bacteriostatic and lytic.[26] He was severely criticized for this view by Mechnikov, who, of course, did not like anyone that believed in the humoral theory of immunity. Not having succeeded in the active vaccination experiment described above, Gabrichevsky attempted to treat his patients by passive immunization. He injected horses with spirochete-containing human blood, and, after an appropriate period of time, prepared an antiserum. He gave this antiserum to his recurrent fever patients during their first apyretic period.

Among the treated patients, 47 percent had no relapses, whereas 37 percent had two, and 13 percent had three relapses. Among the untreated patients, 13 percent had no relapses, 33 percent had two and 47 percent had three relapses. Clearly, Gabrichevsky's experiment was moderately successful. Gabrichevsky's other accomplishments were previously described in this journal.[27]

G.N. Gabrichevsky, researcher on relapsing fever, malaria, diphtheria and scarlet fever, and immunization against them.

An important contribution to the understanding of spirochetal jaundice or Weil's disease was made by N. P. Vasiliev, a privatdozent and an attending physician at the Alexander Hospital in St. Petersburg.[28] Between the years 1883 and 1888 he observed 11 cases of an acute disease characterized by a sudden onset of chills, headache, weakness, excruciating muscular pains, and jaundice. In addition, albumin and various types of kidney cells were observed in the urine. The disease lasted from two to four weeks, and the patients experienced complete recovery. At no time was Vasiliev able to observe any microorganisms in the blood of these patients. He reviewed the observations of all investigators who had described this disease, starting with Landouzy in 1883, Weil in 1886, and his own work. He noted that a total of 48 cases had been described, and his investigation further

showed that the disease was most prevalent during the months of June to August affecting mostly men aged 16 to 24. Vasiliev's paper was also valuable in distinguishing the disease from other similar disorders. He argued against Weil's proposal that the disease was a variation of the typhoid fever, and rejected the idea that it was a form of relapsing fever, as had been proposed by Griesinger. The latter, in 1853, reported the occurrence of a disease in Egypt with symptoms quite similar to those seen by Weil and Vasiliev, and named it *febris recurrens biliosa.* Vasiliev proposed that the disease observed by Griesinger, Weil, and himself be termed *typhus biliosus*, though it should not be confused with any intestinal disorders. Today it is known that Weil's disease is caused by a unique microorganism, *Leptospira icterohaemorrhagiae,* discovered in 1914 in Japan. Vasiliev's contribution to the knowledge of Weil's disease is recognized in some literature which refers to it also as *Vasilieff's disease.*

LEPROSY AND OTHER MYCOBACTERIAL DISEASES

Leprosy was initially studied in Russia by Gregory H. Minkh (Münch) (1836-1896), a professor at Kiev University. The Soviets have claimed that Minkh established the infectious nature of the disease, although it appears that his contribution was confined to epidemiological studies in Southern Russia. A major contribution to the understanding of leprosy was made by V. J. Kedrovsky (1865-1937) of the Pathologic-Anatomical Institute of the Moscow University,[29] who was one of the first scientists to grow the leprosy bacillus in a pure culture. He devised a culture medium, whereby human placenta was homogenized with distilled water, the suspension was filtered through a bacterial filter, and mixed with either peptone broth or agar. Such a medium served well for the culturing of tubercle bacilli, gonococci, and influenza bacilli. He then inoculated agar slants containing this medium with the blood and the extract of a skin nodule of a leprosy patient. Growth was seen in two to five days. The organisms were morphologically identical to authentic leprosy bacilli, though they proved not to be acid-fast. He scrupulously excluded any possibility of contamination, and concluded that the differences from *Mycobacteria leprae* cultured *in vivo* were due to changes that occurred during the *in vitro* culturing of the bacilli. He felt that the characteristics of the leprosy bacilli placed the organism between those of the tubercle bacillus and the diphtheria bacillus.

Tuberculosis received relatively little attention in Russia from the point of view of its fundamental characteristics. The possibility of vaccinating against tuberculosis was brought up as early as 1868 by A. Petrov from Kazan University,[30] who sprayed extract from a tubercular nodule into the pleural cavity of a guinea pig and demonstrated the development of immunity to the disease. Petrov did not follow this with any human experiments. A very ingenious proposal for treating tuberculosis in human beings was put forth by S. Metalnikov from the zoological laboratories of the St. Petersburg Academy of Sciences.[31,32] He had noted that bee larvae required beeswax for proper development, and reasoned that the waxy cell walls of the Mycobacteria might also be digestible by the bee larvae, and that they might thus be immune to the tubercle bacilli. When he injected these bacilli into the larvae, he noted that they were rapidly taken up by the phagocytes and had completely disappeared from the animals within 60 minutes. The larvae were not immune to fish tuberculosis, however. He then purified the mycobacterial wax, and upon injecting it into the larvae, found that it was immediately destroyed. He surmised that there was a specific lipase in the hemolymph of the bee larvae that digested both the besswax and the mycobacterial wax. Metalnikov then proposed that tuberculosis patients might be treatable by the injection of larval blood. To test his hypothesis, he infected guinea

G.N. Minkh, leprosy epidemiologist.

pigs with massive doses of tubercle bacilli and followed this with an injection of larval blood. All the animals getting the larval blood survived, whereas those that did not receive the blood died within one month. It is not known whether or not any attempts were ever made to treat human beings by this method. Metalnikov was a zoologist, and his work on tuberculosis was undoubtedly inspired by another zoologist, Elie Mechnikov, in whose laboratory Metalnikov had spent some time. As is well known, Mechnikov was able to discover phagocytosis at an earlier time by working entirely with insects.

PARASITIC DISEASES

Russian scientists were responsible for much of the early work in the field of parasitic diseases and their mode of transmission. Melnikov, working with Leuckart, was one of the first to suspect the role of insects in the transmission of parasitic diseases. He found that canine tapeworm was introduced into the animals via the dog louse.[33]

A major contributor to our understanding of insect-borne diseases was Alexei P. Fedchenko (1844-1873), a natural sciences graduate of the Moscow University. He was particularly interested in two-winged insects, and compiled an extensive encyclopedia on them. He was also an enthusiastic traveler and explorer, having visited several Siberian and Asiatic regions of Russia for the purpose of gathering specimens. In 1869 Fedchenko investigated the life cycle of a filarian, which he named *Filaria medinensis,* today known as *Dracunculus medinensis.* He showed that the parasite invaded the cyclops, a small tick-like arthropod, whose infected larvae, in turn, found their way into the human or canine body via drinking water. This work was published in 1871 in a local Russian journal for naturalists.[34] In 1872 Fedchenko described the life cycle of the nematode parasite *Gnathostoma hispidum,* which he found mostly in mammals feeding upon cold blooded vertebrates. Unfortunately Fedchenko met an early death in an attempt to climb Mt. Blanc in the Swiss Alps.

Animal parasites were investigated by several Russian scientists, among whom one could mention Wrublevsky and Danilevsky. A trypanosome that invaded the blood stream of the wild bison was discovered by Wrublevsky in 1909.[35] The protozoan was some 30-50μ long, i.e., of intermediate size compared to other known protozoans. It had a well-defined nucleus, centrosomes, and flagellae, and moved rapidly when examined in aqueous suspension under the microscope. Vladimirov and Yakimov confirmed Wrublevsky's findings, and proposed that the new protozoan be named *Trypanosoma wrublevskii.* Danilevsky, a Kharkov University professor, is credited with the discovery of non-pathological blood parasites in birds and reptiles, which he called *Haematozoa.*[36] He also noted the similarity between these animal parasites and the malarial parasite of man, and today these are grouped into a single sub-class *Haemosporina.*

Several years before the appearance of the work of Leischman, Donovan, and Wright on Kala-Azar and the oriental sore in 1903, a Russian military surgeon, Peter F. Borovsky (1863-1932) had already established the causative agent of the oriental sore *(Leishmania tropica)*. Borovsky's work appeared in 1898 in the "Military-Medical Journal," and was not known to the West until Hoare[37] endeavored to write about it in 1938. Borovsky was a graduate of the Military-Medical Academy, and upon graduation in 1891 and thereupon became a staff surgeon and director of bacteriological laboratories at a military hospital in Tashkent. He remained in Tashkent for the rest of his life. Borovsky's studies on oriental sore were started in 1894. He was able to isolate many different microorganisms from the ulcers of the affected patients. However, he felt that all of them except a protozoan 1.5-2μ in diameter were present due to secondary infection. The protozoan observed by Borovsky had a distinct nucleus and multiplied by simple fission or by budding. The protozoa were found in the skin papules of infected individuals and in the early stages of ulcer development. However, the protozoa were absent from older ulcers, where, instead, a multitude of other bacteria were found. He definitely concluded that oriental sore was not caused by bacteria, but was, instead, caused by a higher-order organism, the protozoan. E. J. Marzinovsky and S. L. Bogrov, in a Russian publication in 1904, classified Borovsky's organism as a member of the Trypanosoma family.

Borovsky's work was later extended by his colleague at Tashkent, K. Shulgin, who, in 1902, in a Russian publication, reported on the mode of transmission of the oriental sore. In eight cases, he was able to establish that the development of the oriental sore was preceded by mosquito bites. He also noted that the disease made its appearance during late summer and early fall, and that the disease among army personnel was confined to enlisted men, and was practically absent among the officers, who customarily slept under mosquito netting. All these facts indicated to Shulgin that the mode of transmission of oriental sore was via insect bites.

Malaria was quite prevalent in Russia. Gabrichevsky (see above) was one of the more active malaria fighters, who participated in many expeditions to the remote areas of Russia infested by the malaria parasite. The commission in which Gabrichevsky participated, recommended the prophylactic use of quinine and the use of mosquito netting. Where these simple recommendations were implemented, the incidence of malaria was reduced two-to-three-fold.[38] The most distinguished Russian malaria researcher was Dimitry Romanovsky (1861-1921), who elucidated the life-cycle of the malarial parasite.[39] He was born in the city of Pskov, and graduated from the Military-Medical Academy in 1886. In 1891 he was awarded the doctorate in medicine for his discovery of what we today call the Romanovsky stain and for the use of this stain to study the malarial parasite.

Romanovsky's stain was prepared by titrating a saturated solution of methylene blue with a one-percent aqueous solution of eosin until a violet precipitate appeared. This was generally accomplished when one part of the methylene blue solution was mixed with two parts of the eosin solution. Romanovsky's dye stained all nuclei dark-purple, whereas the red cells were stained pink and the platelets appeared dark red-to-violet. Eosinophile leukocyte protoplasm stained intensely pink, whereas that of the polynuclear neutrophilic leucocytes stained light violet with dark violet coloration in the granules. The protoplasm of both the lymphocytes and the mast cells stained intensely blue. Spirochetes in blood were also stained blue.

The malarial parasite itself showed remarkable detail when stained with the Romanovsky dye. He found two types of structures in the cell: a blue-staining area and a colorless area, which contained purple granules. He recognized the latter as being the parasite nuclei. On the basis of his microscopic examination, Romanovsky was able to divide the life cycle of the

malarial parasite into four stages: the first stage involved extracorpuscular parasites, which soon were seen to send out protrusions which punctured holes in red cells in preparation for entry of the parasite. After the parasite entered the red cell, the second stage began with the parasite growing to a size almost as large as the entire red cell. The mature intracorpuscular parasite represented the third stage of development. Here it began to form chromatin network in its nucleus, and the parasite cell divided into as many as 20 daughter parasites per single red cell. The red cell then burst and released the parasites, which searched out new victims among the red cell population. Rarely, sporulating forms of the malarial parasite were observed by Romanovsky, and to these he assigned the fourth stage of development. The editors of the "St. Petersburger Medicinische Wochenschrift," where Romanovsky's paper was published, prefaced his article with a note predicting that his method would be extremely useful for the recognition of malaria and its stage of development in clinical practice. This proved to be an understatement, since Romanovsky's method is today used not only for the staining of blood smears, but is also the basis of a number of staining techniques in pathological examinations of tissue.

OTHER INFECTIOUS DISEASES

The causative agent of erysipelas in man was established in 1887 in St. Petersburg by Meierovich.[40] In his doctoral dissertation, Meierovich reported isolating the erysipelas streptococcus from the skin and blood of several patients. The microorganisms grew rapidly in conventional broth media either at room temperature or at 37°, and these cultures were used to transmit the disease to rabbits. Those rabbits that did not survive the disease showed microorganisms in their blood and many organ systems. Those who survived were immune to re-infection for one to two months after the disease. Meierovich called attention to the fact that several types of microorganisms could cause erysipelas; however, he appeared to consider *Streptococcus pyogenes* as the principal offender. *Streptococcus pyogenes* was identified as the causative agent of erysipelas also by A. Pavlovsky,[41] who pointed out that this microorganism was responsible for the less serious type of erysipelas, whereby purulent discharge or gangrene were not observed in rabbits infected with the agent.

Fundamental work on the pathogenesis of *Enterobacteriaceae* may be exemplified by the contributions of L. Rosenthal of Gabrichevsky's Bacteriological Institute in Moscow. Shortly after the discovery of the dysentery bacillus by Shiga, Rosenthal was able to isolate the microorganism from his patients, and to show that they did not generally invade the blood stream. He found agglutinating antibodies in the sera of convalescent patients on the 10th to 12th day of the illness. He even tried to immunize guinea pigs with the microorganisms, though this did not meet with much success.[42] Rosenthal was also successful in preparing the dysentery bacillus toxin by growing the microorganism in a conventional slightly alkaline broth for three weeks. A 0.1 ml aliquot of the culture filtrate was able to kill a 2 kg rabbit. The toxin was precipitable with ethanol, and could be redissolved in a weak salt solution. It was heat and acid stable.[43] Rosenthal then used the toxin to produce an antiserum in horses, and used this serum to treat his dysentery patients. Of the 157 patients treated, only eight, i.e., 4.5 percent did not survive. The death rate among the untreated patients was 12.2 to 17.7 percent. In addition, the hospital stay of the treated was, on the average, ten days, against 16 days for the untreated patients.[44]

An energetic advocate of blood culture for diagnostic purposes was N. Klodnitsky, a bacteriologist with the Interior Ministry Bacteriological Laboratories in Astrakhan.[45,46] He was convinced that red cells had bacteriocidal properties, and proposed that, for bacteriological examina-

tion, blood should be drawn and immediately mixed with distilled water in a ratio of 0.5 to 4.5 ml to lyse the cells and to dilute the humoral antibodies. This dilution was then to be inoculated into agar or nutrient broth. Using his method, Klodnitsky was able to obtain cultures from the blood of patients suffering from typhoid, gonorrhea, paratyphoid A and B fever, pneumococcal pneumonia, and streptococcal and staphylococcal septicemia.

CONCLUSION

It is seen from the preceding narrative that Russian physicians and microbiologists of the pre-World War I era were responsible for a number of advances in the area of infectious disease therapy and understanding of its etiology. There was both the talent and desire among the members of Russia's academic community to participate in the eradication of infectious diseases in Russia. Yet progress was too slow, probably because of the largely rural nature of the populace, under-industrialization of the country, a shortage of medical facilities, and the ineptness of many officials. The work of Russia's microbiologists was, of course, not restricted to agents of infectious disease. There were numerous investigators, such as Vinogradsky, Mechnikov, Gamaleya, Omeliansky, Nadson, etc., who made fundamental contributions to our understanding of bacterial physiology and to immunity. Their work will, however, be discussed in another paper at a later time.

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ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Anesthesiology

Sadove MS, Kim SI: *Hepatitis after use of two different fluorinated anesthetic agents. Anesth Analg 53:336, 1974*

A patient who had been previously anesthetized with halothane developed hepatitis following a second operation, a month later, for which another fluorinated anesthetic agent, enflurane, was used. The clinical syndrome was almost identical to that of "halothane hepatitis." A possible mechanism is discussed, although no conclusion is reached. However, the authors wish to alert their confreres to the risk of hepatitis when two successive anesthetic procedures employ fluorinated agents, even if these are different products.

Biomechanics

Hardison WGM, Apter JT: *Use of hybrid computers to analyze behavior of detailed models of biological systems. II: System parameters used in bile salt stimulated biliary cholesterol excretion. Comput Biol Med 4:3, 1974*

A hypothesis relating biliary bile salt excretion and cholesterol and phospholipid excretion was formalized into a model which could be programmed on an analog computer. Computer curves were generated by entering into the program bile salt infusion rates, parameters derived experimentally for each animal, and certain constants derived from the data. These curves closely approximated the measured biliary cholesterol and phospholipid outputs when other system parameters of the model were scanned systematically with a medium-sized digital computer. In this way the model served as a suitable framework for identifying the biological significance of the model parameters.

Biochemistry

Bezkorovainy A, Grohlich D: *Comparative study of several proteins of the transferrin class. Comp Biochem Physiol 47:787, 1974*

Human, baboon and bovine serum transferrins, hen's egg conalbumin, and human milk lactotransferrin were treated with cyanogen bromide, and proteins thus fragmented were subjected to N-terminal group analysis and polyacrylamide gel electrophoresis containing mercaptoethanol, urea and sodium dodecyl sulfate.

2. The human and baboon transferrins were each split into six fragments; bovine transferrin gave seven to eight fragments, conalbumin gave eight fragments, and lactotransferrin was split into only five fragments.

3. The fragments obtained from human, baboon and bovine transferrins had similar molecular weights, ranging from 28,000 to 9,000, whereas lactotransferrin was different in that its two major fragments had molecular weights of 45,000 and 9,000.

4. It was concluded that the transferrins studied, other than lactotransferrin, probably do not contain sections of identical structure, and that lactotransferrin may represent a useful model for the study of the origin of the transferrins.

Bezkorovainy A, Zschocke RH: *Structure and function of transferrins, physical, chemical, and iron-binding properties. Arzneim Forsch (Drug Res) 24:476, 1974*

The transferrins are a group of homologous, non-heme ironbinding proteins. They are found in the blood of vertebrates, in milk and other secretions of mammals, and in the egg-white portion of avian eggs. The present discussion provides a detailed account of the more recent advances made toward the understanding of the physical, chemical, and iron-binding properties of these proteins. Much of what is known about the transferrins today has come from the intensive investigation of human serum transferrin, human lactoferrin, and hen's egg conalbumin. More recently, studies have been extended to transferrins from other species, and comparative data are included.

All transferrins studied so far bind two atoms of iron (as Fe^{+3}) per protein molecule. The iron is bonded to two apparently identical, though independent, binding sites via amino acid side-chains of the polypeptide backbone. There is now general agreement as to the identity of the amino acids involved. In addition, bicarbonate (carbonate?) is an essential ligand in the iron-transferrin complex. There are indications that the binding sites may differ with respect to the way in which bicarbonate is ligated to the complex. Also, there is evidence from physical and chemical studies suggesting that the binding sites do differ from each other. This lends new support to the earlier hypothesis that the two binding sites of serum transferrin assume different physiologic functions, which are discussed in the second part of this review.

In spite of the presence of two active centers in the molecule, the transferrins are devoid of a subunit structure. Therefore, and considering the apparent identity of the binding sites with respect to the amino acids involved in iron-binding and the individual binding constants observed, it was assumed that the polypeptide chains of these proteins may consist of two fused, identical halves. Most evidence available now, however, does not substantiate this assumption, nor does it indicate the presence of larger repeating segments within the polypeptide chain of the transferrins.

Like many other proteins, most transferrins exhibit genetic polymorphism, which is determined by independent allelic genes and which, on a molecular basis, finds its expression in amino acid substitutions in the polypeptide chain.

Miyabo S, Kornel L: *Corticosteroids in human blood. VI: isolation, characterization and quantitation of sulfate conjugated metabolites of cortisol in human plasma. J Steroid Biochem 5:233, 1974*

Evidence has been obtained for the presence of a total spectrum of sulfate-conjugated metabolites of cortisol in human plasma: 19 monosulfates, two disulfates and six glucuronsulfates. Two hours and 15 min following i.v. administration of a tracer dose of ($4^{-14}C$)-cortisol to 11 normal subjects, large samples of heparinized blood were obtained. From the separated, deproteinized and defatted plasma, all steroids were extracted by means of an XAD-2 amberlite column. Mono- and di-sulfate conjugated steroids were then separated from other free and conjugated metabolites of cortisol by means of high voltage paper electrophoresis. Individual monosulfate conjugated metabolites and glucuronosulfates were subsequently separated (as seven sub-groups) by paper chromatography. These conjugates were eluted and subjected to cleavage by solvolysis (monosulfates and disulfates) or consecutive solvolysis and β-glucuronidase hydrolysis (glucuronosulfates). The liberated steroid moieties were separated by multiple successive paper chromatographies, and identified by reverse isotope dilution technique. The individual steroids were then quantitated. Sulfate-to-steroid molar ratios were also determined on major conjugates, prior to their cleavage.

The steroid metabolites found in plasma are identical with those isolated by us previously from human urine. The total sulfate fraction constituted 5•3 ± 1•9 percent of all conjugated metabolites of cortisol in plasma. Of those, 76 percent were monosulfates, 19 percent were glucuronosulfates, and 5 percent were disulfates. A large proportion of monosulfates consisted of steroids conjugated at C-21 (27 percent), which contrasts with

glucuronide conjugates. The steroids found in largest concentrations were: 20β-cortolone-3-sulfate (17 percent), 5α-tetrahydrocortisol-3-sulfate (14 percent), 6β-hydroxycortisol-21-sulfate (9 percent), cortisol-21-sulfate (8 percent), tetrahydrocortisol-3-sulfate (7 percent), and 20β-dihydrocortisol-21-sulfate (6 percent).

From the correlation of plasma and urinary concentration of individual steroid sulfates, the following conclusions are drawn: (1) sulfoconjugation is a consistent, though quantitatively minor, pathway in the peripheral metabolism of cortisol; (2) C-21 sulfates of 4-ene-3-oxo steroids are formed (and excreted) considerably faster than C-3 sulfates, the formation of which requires a prior reduction of a ring-A; (3) C-3 sulfates of steroids with dihydroxyacetone side-chain are formed (and excreted) more slowly than the corresponding sulfates of steroids with glycerol side-chain, *ergo*, the reduction of 20-ketone appears to facilitate sulfoconjugation.

Nichols JH, Bezkorovainy A: *Human colostral whey M-1 glycoproteins and their* L. bifidus var. Penn. *growth promoting activities. Life Sci 14:967, 1974*

A total of seven M-1 glycoprotein fractions were isolated from human colostrum whey and all showed *L. bifidus var. Penn.* growth promoting activities using well defined chemical medium. The growth promoting activity of these fractions was parallel to their total carbohydrate content. On the other hand, bovine colostrum M-1 glycoprotein had a relatively low growth promoting activity, whereas human serum orosomucoid had none.

Springer GF, Adye JC, Bezkorovainy A, Jirgensons B: *Properties and activity of the lipopolysaccharide-receptor from human erythrocytes. Biochemistry 13:1379, 1974*

We have isolated from human erythrocyte membranes a physicochemically homogeneous lipoglycoprotein with a molecular weight of 256,000. It is rich in *N*-acetylneuraminic acid, galactose, and hexosamines. The intact substance prevented attachment to erythrocytes of unheated and heated, smooth and rough lipopolysaccharide and protein-lipopolysaccharide of all gram-negative bacteria tested. It did not interact with other bacterial antigens and therefore is referred to as "lipopolysaccharide-receptor." The receptor physically and reversibly blocked those groupings on lipopolysaccharide which attach to red cells. Both citraconylation and dissociating polyacrylamide gel electrophoresis under standard conditions produced one large and one small fragment. Citraconylation-inactivated decitraconylation of the large fragment restored high activity but the small fragment remained inactive. Only the large fragment had a composition similar to the intact receptor. The light fragment had significantly less apolar amino acids and carbohydrate. Prolonged incubation of the small fragment in sodium dodecyl sulfate resulted in its further fragmentation. Circular dichroism spectra of the intact lipopolysaccharide-receptor showed α-helix, β-conformation and some flexible aperiodic conformation. Removal of lipid resulted in extensive disorganization. Receptor activity was destroyed by proteases; lipid and carbohydrate were not involved in the receptor's activity.

Bronchoesophagology

Holinger PH, Schild JA: *Anomalies of esophagus.* In: *Gastroenterology, 3rd Ed, Vol 1, ed. by Henry L. Bockus, 1974, Philadelphia, W. B. Saunders Co., pp. 183-190*

Congenital malformations of the esophagus are most frequently detected in the first few days of life as immediate regurgitation, choking and cyanosis recur with each attempt at nursing soon after birth. However, occasionally esophageal anomalies are found responsible for dysphagia, dyspnea, recurrent pneumonia or unexplained vomiting during childhood or even in adult years. Advances in radiologic diagnosis through the use of

image intensifiers and cinefluoroscopy have demonstrated a surprising frequency of some anomalies, previously considered rare, as, for example, the "H" fistula. Surgical correction of many of these conditions has kept pace with their increased recognition, proving the importance of accurate diagnosis. In this chapter, a classification of the congenital anomalies is suggested which includes the atresias, webs, anomalies produced by external compression, duplication, neurogenic abnormalities and other anomalies such as the sequestrated lung arising from the esophagus. Illustrations by means of x-rays are utilized throughout this chapter.

Dermatology

Pearson RW, Potter B, Strauss F: *Epidermolysis bullosa hereditaria letalis: clinical and histological manifestations and course of the disease. Arch Dermatol 109:349, 1974*

Five cases of epidermolysis bullosa hereditaria letalis are presented as a basis for discussion of the clinical features and course of the disease. The distribution and morphologic characteristics of the lesions suggest the diagnosis, but histologic findings, and particularly the ultrastructural findings, are crucial to early diagnosis. Blisters form between the plasma membrane of the basal cell and the basement membrane, but the diagnosis can be made from examination of the epidermis alone. The microscopical findings suggest several possibilities concerning the nature of the basic defect; the most plausible is that the junction is under attack by lytic enzymes. Most patients live only a few weeks or months, but patients who do survive retain distinct clinical and microscopical features that allow easy differentiation from other mechanobullous diseases.

Genetics and Human Development

Garron C, Lindsten J: *Sex ratio among normal sibs of persons with Turner's syndrome. Clin Genet 5:406, 1974*

Several reported samples of persons with Turner's syndrome are combined, and the sex ratio among their normal sibs calculated. There is a tendency for an excess of males among the sibs of both XO and non-XO probands, in contrast to an excess of females in a relevant general population and in a selected control sample. This high sex ratio is consistent with the maternal origin of the X chromosomes in the majority of XO probands, and with randomness in the loss of parental sex chromosomes, in the combination of resulting gametes, and in the non-viability of OY zygotes.

Gynecologic Oncology

Shingleton HM, Wilbanks GD: *Fine structure of human cervical intraepithelial neoplasia* in vivo *and* in vitro. *Cancer 33:981, 1974*

The fine structure of normal cervical squamous cells and cells of cervical intraepithelial neoplasia (CIN) from both biopsies and tissue culture was studied. The cultured CIN cells were found to retain their epithelial characteristics even after subculturing and to resemble in most aspects their *in vivo* counterparts; they were found to differ markedly from cultured normal squamous cells. It was not possible to distinguish "dysplasia" from "carcinoma-in-situ" at the ultrastructural level in either biopsies or cultured cells.

This lends support to the concept that the two are part of a continuum, not separate entities. It would appear that cultures of normal cervical squamous cells and CIN cells may be useful for further study of the biology of cervical neoplasia.

Hematology

Cole ER: *Effect of steroids, including progestin and estrogen components of oral contraceptive drugs, on the esterase activity of thrombin. Thromb Res 4:551, 1974*

TAME esterase activities of bovine and human thrombin are accelerated in the presence of certain steroids. Steroids of the androstane series are the most active, those of the pregnane series are less acceleratory, and the natural estrogens are not acceleratory. However, some progestin and estrogen analogs, utilized as components of oral contraceptives, significantly increase the TAME esterase activity of thrombin. The conformation of the steroid nucleus and the nature and configuration of substituent groups are important in determining whether a steroid is acceleratory or non-acceleratory in the thrombin esterase assay system.

Knospe WH: *Malignant lymphoma: staging and classification. Postgrad Med 55:201, 1974*

Accurate staging of malignant lymphoma is the guide to optimal treatment. The most recent classification system, the Ann Arbor staging system, allows distinction by both clinical and pathologic stages, permitting a precise definition of the extent of lymphomatous disease both before and after staging laparotomy. The histologic category of Hodgkin's disease has important implications referable to agressiveness of the lymphomatous process and may have predictive value in terms of survival.

Knospe WH, Loeb V Jr, Huguley CM Jr: *Bi-weekly chlorambucil treatment of chronic lymphocytic leukemia. Cancer 33:555, 1974*

Because the lymphocytes of chronic lymphocytic leukemia (CLL) are known to proliferate slowly, it was postulated that intermittent therapy might have a cumulative inhibitory effect on tumor cells while permitting normal cells to recuperate between doses. Sixty-two evaluable patients with CLL were treated with chlorambucil given orally as a single pulse every two weeks. The initial dose was 0.4 mg/kg; subsequent doses were increased by 0.1 mg/kg until toxicity or disease control was achieved. Responses were obtained in six of eight (75 percent) previously untreated patients with indolent disease, in 18 or 31 (61 percent) previously untreated patients with active disease, in 7 of 14 (50 percent) previously treated patients not shown to be resistant to alkylating agents, and in two of nine (22 percent) patients resistant to prolonged daily chlorambucil therapy. The over-all effectiveness in patients with CLL not previously resistant to chlorambucil was 31 of 53 (58 percent), with five complete remissions (9 percent). Hematologic toxicity was usually mild and never life-threatening. Gastrointestinal toxicity, which occurred in 23 of 62 patients, was usually mild and easily controlled with antiemetics. It is concluded that bi-weekly oral administration of chlorambucil is effective therapy for CLL with response rates similar to daily continuous chlorambucil. Hematologic toxicity is considerably less than with daily treatment.

Edwards LD, Cross A, Levin S, Landau W: *Outbreak of a nosocomial infection with a strain of* proteus rettgeri *resistant to many antimicrobials. Am J Clin Pathol 61:41, 1974*

Over a three-month period, *Proteus rettgeri* infections occurred in ten patients on one general medical ward. This organism was resistant to all antimicrobials tested by the Bauer-Kirby and tube dilution methods including tobramycin, gentamicin, kanamycin, carbenicillin, ampicillin, cephalothin, tetracycline, chloramphenicol, polymyxin and rifampin. Clinical illness from this organism was directly related to local or systemic host deficiencies. Typically, patients were chronically ill with recent urinary tract instrumentation and infection and previous antimicrobial therapy. Two immunosuppressed patients became infected, one after renal transplantation. Tissue invasion was demonstrated in three patients by the occurrence of a subcutaneous abscess, renal abscesses, and bacteremia, respectively. Aside from the patients, no reservoir was shown by culturing personnel, catheter kits, the hospital environment, and the stools and urine of every patient on the ward. Probable spread was by contact through the intermediary of hospital personnel. Control was obtained by having personnel use disposable gloves and contact isolation of patients. The importance of this organism is stressed by the fact that no therapeutic agent was effective *in vitro* and *in vivo.*

Nelson KE, Kallick CA, Resnick L, Kallick S, Gotoff SP, Levin S: *Current strategy for urban measles control. JAMA 227:780, 1974*

Reported measles attack rates are substantially less since licensure of vaccine. Nevertheless, measles continues to be an important cause of morbidity among inner-city populations. In an urban epidemic that occurred after vaccine licensure, the deaths, encephalitis cases, and complication rates among hospitalized patients were similar to those in a prevaccine epidemic. In the earlier and later epidemics, respectively, 23.2 percent and 30.1 percent of hospitalized patients were less than one year old. In the later epidemic, attack rates were much greater in lower socioeconomic areas than in higher ones.

Vaccine failure did not contribute greatly to the later epidemic. Childhood measles vaccination should be given high priority. As long as measles risk remains high, vaccination appears indicated for infants six to nine months old from crowded, lower-income urban areas. These infants will need booster doses later to ensure immunity.

Microbiology

Deinhardt F: *Oncogenic herpesviruses in species other than owl monkeys. J Med Prim 3:79, 1974*

Two herpesviruses, Epstein-Barr virus of man and *Herpesvirus saimiri* of squirrel monkeys, are very similar in their expression, both in their natural and experimental hosts. Study of their respective roles in inducing lymphomas, leukemias and other lymphoproliferative diseases, and most particularly study of *Herpesvirus saimiri* in nonhuman primates, has increased understanding of the pathogenesis of malignant disease.

Deinhardt F, Falk LA, Wolfe LG: *Transformation of nonhuman primate lymphocytes by Epstein-Barr Virus. Cancer Res 34:1241, 1974*

Continuous lymphoblastoid cell cultures were established from marmoset *(Saguinus sp.)*, squirrel *(Saimiri sciureus)*, owl *(Aotus trivirgatus)*, and cebus *(Cebus apella)* monkeys after their peripheral lymphocytes were cultured with lethally, X-irradiated cells carrying Epstein-Barr virus (EBV). Simian lymphocytes were also transformed after exposure to infectious, cell-free EBV derived from some simian lymphoblastoid cell cultures. EBV-induced early, viral capsid, and membrane antigens, intranuclear inclusion bodies, and herpesvirus virions were demonstrable in most cell cultures. All cell cultures had B-cell characteristics; they produced immunoglobulins but did not form spontaneous rosettes with sheep erythrocytes. Four of six marmoset monkeys inoculated with EBV-transformed marmoset lymphocytes developed antibodies to Epstein-Barr viral capsid antigens, one marmoset inoculated with autochthonous transformed cells developed heterophile antibodies, and one of five marmosets inoculated with cell-free EBV developed a lymphoma. No overt clinical abnormalities were detected in any of the inoculated marmosets.

Falk LA, Wolfe LG, Deinhardt F: *Experimental infection and lymphoma induction in marmoset monkeys. Fed Proc 33:Mar 1974*

Marmoset monkeys were inoculated with various preparations containing EBV: (1) cell-free virus from EB3 and HR-1 cell cultures, (2) autologous and allogeneic lymphocytes transformed by EBV *in vitro*, or (3) cell-free virus from a marmoset lymphoblastoid cell line, B95-8, transformed by EBV *in vitro* (Miller *et al.* PNAS 69:383-387, 1972) and oncogenic *in vivo* (Shope *et al.* PNAS 70:2487-2491, 1973). No disease was observed in marmosets inoculated with cell-free virus from EB3 or HR-1 cultures or with autologous or allogeneic EBV-transformed lymphocytes; marmosets inoculated with transformed cells and virus from B95-8 cultures developed antibodies to EBV-determined antigens. One of five marmosets inoculated with B95-8 virus developed malignant lymphoma and died 31 days PI; lymphoblastoid cell cultures were established from lymph node and spleen of this marmoset and also of a marmoset that died 19 days PI with no evidence of disease. The cell cultures established from both animals expressed EBV-specified viral capsid, early, nuclear (complement dependent) and membrane antigens; IgG and IgM immunoglobulins were produced by the cells but rosettes were not formed with sheep erythrocytes. These studies further indicate that marmoset monkeys may serve as models for studying the oncogenic potential of EBV and immune responses to EBV infection.

Falk LA: *Oncogenic DNA viruses of nonhuman primates: a review. Lab Anim Sci 24:182, 1974*

Studies of experimental infection of nonhuman primates with four members of the herpesvirus group were reviewed. *Herpesvirus saimiri* and *Herpesvirus ateles*, indigenous in *Saimiri sciureus* and *Ateles sp*, respectively, caused no recognized disease in the natural hosts but caused fatal, malignant lymphoproliferative disease in other nonhuman primate species. Epstein-Barr virus (EBV) and Herpesvirus homonis (HVH) of man have been implicated as the etiologic agents of several human neoplasms. Recent studies indicate that nonfatal EBV and HVH infections can be established in certain nonhuman primate species, and several features of these infections, i.e., antibody formation and shedding of virus, resemble latent infections in humans that are caused by EBV and HVH.

Falk L, Wolfe L, Deinhardt F, Paciga J, Dombos L, Klein G, Henle W, Henle G: *Epstein-barr virus: transformation of non-human primate lymphocytes* in vitro. *Int J Cancer 13:363, 1974*

Continuous lymphoblastoid cell cultures were established from marmoset *(Saguinus sp.),* squirrel *(Saimiri sciureus),* owl *(Aotus trivigatus)* and cebus *(Cebus apella)* monkeys after culturing their peripheral lymphocytes with lethally X-irradiated cells carrying Epstein-Barr virus (EBV). Transformation also was achieved by exposing simian lymphocytes to infectious, cell-free EBV derived from the simian lymphoblastoid cell cultures. Simian lymphocytes were not transformed after exposure to cell-free EBV derived from HR-1 cells. The simian cell cultures were similar to cell cultures derived from Burkitt's lymphoma or infectious mononucleosis patients. EBV-induced early, viral capsid and membrane antigens, intranuclear inclusion bodies and herpesvirus virions were demonstrable in most cultures. Seven cultures were insusceptible to superinfection with EBV and treatment of the cultures with halogenated pyrimidines was relatively ineffective for inducing synthesis of early or viral capsid antigens. All cell cultures had B-cell characteristics: they produced immunoglobulins but did not form spontaneous rosettes with sheep erythrocytes. Four of six marmoset monkeys, inoculated with EBV-transformed marmoset lymphocytes, developed antibodies to EB viral capsid antigens and one marmoset inoculated with autochthonous transformed cells also developed heterophile antibodies. Seven marmosets, inoculated with cell-free EBV derived from HR-1 cell cultures, developed no detectable levels of antibodies to EBV-specified antigens or heterophile antibodies. No overt clinical abnormalities were detected in any of the marmosets inoculated with HR-1 or Kaplan EBV but one of five marmosets inoculated with B95-8 EBV developed a lymphoma.

Hampar B, Tanaka A, Nonoyama M, Derge JG: *Replication of the resident repressed Epstein-Barr virus genome during the early S phase (S-1 period) of nonproducer Raji cells. Proc Nat Acad Sci USA 71:631, 1974*

Replication of the resident repressed Epstein-Barr virus genome in synchronized nonproducer Raji cells was shown to occur during the early S phase (S-1 period) by hybridization of cell DNA with virus-specific complementary RNA (cRNA). The S-1 period was previously identified as the critical period for virus activation induced by thymidine analogues. The findings reported here and elsewhere are consistent with the proposal that: *(i)* virus activation is initiated at or near the site of association of the resident viral genome with cell DNA, *(ii)* replication of the resident virus genome in nonactivated cells is under cell control mechanisms, and *(iii)* the resident virus genome is physically associated with early replicating cell DNA.

Massey RJ, Johnson DR, Odgen J, Deinhardt F: *Production and reactivity of an antiserum to marmoset derived lymphocytes. Fed Proc 33:811, 1974*

Marmoset lymphocytes transformed by *Herpesvirus saimiri* (HVS) behave as thymus derived cells (1-cells) because they form rosettes with sheep erythrocytes and lack surface immunoglobulins. Whereas, lymphocytes transformed by Epstein-Barr virus (EBV) behave as B-cells because of surface immunoglobulins detectable by membrane immunofluorescence. An antiserum was prepared against marmoset *(Saguinus sp.)* T lymphocyte antigen (anti-STLA) by hyperimmunizing a goat with a lymphoblastoid cell line (LCL) established from the thymus of an HVS-infected marmoset. This antiserum

was absorbed with normal marmoset fibroblasts and an immunoglobulin producing LCL established by transforming marmoset lymphocytes with EBV *in vitro*. In serum cytotoxicity and membrane immunofluorescence assays the antiserum reacted specifically with T-LCL but not with immunoglobulin positive B-LCL's and the reactivity could be removed by absorption with thymus tissue. The anti-STLA serum reacted with 70 percent of purified peripheral blood lymphocytes from normal marmosets in a double label membrane immunofluorescence assay, while the remaining 30 percent stained with an anti-immunoglobulin serum. A cross reacting antigen on a human T-LCL was found with this anti-serum, whereas human B-LCL's were negative for this antigen. This anti-STLA serum is being used to evaluate T-cell mediated immunity in marmosets and to immunosuppress the animals' response to virus-induced tumors.

Nigida SM, Falk LA, Wolfe LG, Deinhardt FW, Long WK, Alford CA: *Cytomegalovirus: experimentally-induced infection in marmoset monkeys with a human strain (Colburn). Abst Ann Mtg Am Soc Micro, 1974, p. 216*

Colburn strain of cytomegalovirus (CMV), isolated from a brain biopsy of a child with clinical encephalopathy (Charmamella *et al.*, Abst. Ann. Mtg., ASM, 1972, p. 256), replicates in nonhuman fibroblast cell cultures and differs antigenically from prototype strains. Two adult and two neonatal cotton-topped and two neonatal white-lipped marmoset monkeys *(Saguinus sp.)* were inoculated with 10^7 PFU of Colburn virus. Blood was collected at seven to fourteen day intervals for hematologic and serologic studies and for virus isolation. No overt clinical disease developed in five marmosets during five months' observation; one adult died 63 days post-inoculation (PI) from non-specific causes. All marmosets developed serum antibodies seven to fourteen days PI which were detectable by neutralization and immunofluorescence tests with titers of 1:8-1:16 and 1:64-1:256 respectively. Attempts to isolate virus from peripheral lymphocytes and oropharyngeal or vaginal swabs by cocultivation of permissive cell cultures were unsuccessful. Virus was recovered, however, by cocultivation, from the kidney of the animal which died. The establishment of latent CMV infection in marmosets offers a potential model for further characterization of such type infections in man.

Wright J, Falk LA, Deinhardt F: *Induction of Epstein-Barr virus nuclear antigen (EBNA) in human cord blood lymphocytes. Abst Ann Mtg Am Soc Micro 1974, p. 222*

A complement-dependent Epstein-Barr virus (EBV) nuclear antigen was recently identified by immunofluorescent techniques in EBV-carrying lymphoblastoid cell lines; EBNA was present in all cell lines regardless of whether viral capsid or early antigens (VCA, EA) were expressed (Reedman B, Klein G: Int J Cancer 11:499, 1973). We studied the induction of EBNA in human cord blood lymphocytes infected *in vitro* with EBV to assess the relationship between EBNA and cell transformation. Lymphocytes were infected with EBV obtained from an EBV-producing cell line; as early as one day after infection a few EBNA-containing cells were observed in 9 of 11 infected lymphocyte samples, by day 12 approximately 50 percent of the cells contained EBNA, and within 18 to 24 days 85 to 90 percent of lymphocytes expressed EBNA, and cell transformation was evident. EA was detected in one of six transformed cultures but VCA was not observed. Appearance of EBNA and cell transformation were prevented by inactivating or neutralizing viral infectivity by heat or serum containing EBV antibodies respectively, but not by serum containing antibodies to other herpesviruses. Culture media from cell lines not producing EBV or cell lines carrying *Herpesvirus saimiri* or *Herpesvirus ateles* failed to induce EBNA. These studies indicate that EBV induces EBNA and that appearance and persistence of EBNA accompanies cell transformation.

Neurology

Becker FO, Michael JA, Davis FA: *Acute effects of oral phosphate on visual function in multple sclerosis. Neurology 24:601, 1974*

The acute effects of an oral hypocalcemic substance, NA_2 HPO_4/KH_2 PO_4, was tested on visual function in six multiple sclerosis patients with optic nerve dysfunction. Marked improvement in visual function testing occurred in five patients within one hour after phosphate ingestion and generally reversed over one to two hours. Improvement was correlated with elevation of serum phosphate and chemical and clinical evidence of serum calcium lowering. Placebo had no effect on visual function in one multiple sclerosis patient, and phosphate had no effect on pupillometry in a normal control subject. Though the results do not represent a practical treatment, they do show the dramatic potential for acute pharmacologic modification of signs and symptoms of multiple sclerosis.

Orthopedic Surgery

Rostoker W, Galante JO, Shen G: *Some mechanical properties of sintered fiber metal composites. Test Eval 2:107, 1974*

Kinked, short-length, fine wire can be molded by conventional powder metallurgy procedures and sintered to a porous composite with large proportions of interconnecting voids. This material has potential applications for implanted prosthetic systems. The material behaves in a nonlinear elastic fashion which may be approximated as two linear elastic processes. In the strain range of 0 to about 0.5 percent, the elastic modulus can be less than 1 kg/mm^2. In a higher strain regime the elastic modulus is about 100 kg/mm^2. A total elastic strain range of 1.5 to 4 percent is found.

Pediatrics

Cunningham DC: *Clinical variations of cystic fibrosis. Ill Med J 145:493, 1974*

A brief review of the clinical manifestations of cystic fibrosis is presented with illustrative case presentations. It is emphasized that the sweat test is the only consistent diagnostic tool at the physician's disposal. The indications for sweat testing are discussed from a clinical point of view.

Lange CF, Justice P, Smith GF: *Milk precipitins in mongolism. Res Commun Chem Pathol Pharmocol 7:605, 1974*

Serum of 2,218 individuals was evaluated for precipitins to milk proteins. Of the 2000 normal sera tested 3.8 percent showed positive precipitins, whereas the incidence was 18.7 percent in mongols and 4.7 percent in non-mongol retarded individuals. Only antibodies versus albumin, gamma globulin or both but not to any other milk proteins were

detected in any positive sera. These findings seem to enhance the concept of a generalized increase in immune sensitivity in the mongol.

Pharmacology

McCloy RB, Prancan AV, Nakano J: *Effects of acetaldehyde on the systemic, pulmonary, and regional circulations. Cardiovasc Res 8:216, 1974*

The acute haemodynamic effects of acetaldehyde were studied in anaesthetized dogs. Acetaldehyde increased heart rate, systemic arterial pressure, pulmonary arterial pressure, cardiac output, systemic venous return, and myocardial contractile force. Acetaldehyde constricts the peripheral vascular beds in the brachial, femoral, renal, and common carotid arteries and dilates those of the coronary, hepatic, and superior mesenteric arteries. Alpha- and beta-adrenergic blockade not only abolished the cardiovascular effects of acetaldehyde, but also reversed its pressor effect. It is concluded that most of the haemodynamic effects of acetaldehyde result from the release of catecholamines from sympathetic nerve endings and the adrenal medulla.

Willerson D Jr, Kass L, Frischer H, Bowman JE, Rieckmann KH, Carson PE, Richard L: *Chemotherapeutic results in a multi-drug resistant strain of* plasmodium falciparum *malaria from Vietnam. Milit Med 139:175, 1974*

This report presents the results of chemotherapeutic studies with a strain of *Plasmodium falciparum* from Vietnam. This strain has been termed the Vietnam (Marks) strain.

Fifty-nine volunteers participated in chemotherapeutic studies involving a strain of *P. falciparum* isolated from a soldier serving in Vietnam. Radical cure of acute *P. falciparum* infections in non-immune volunteers was most often achieved following treatment using combined drug regimens, including trimethoprim and sulfalene (five of seven volunteers), quinine and tetracycline (five of five volunteers), or quinine and sulfalene (two of two volunteers).

SUGGESTIONS TO AUTHORS

Manuscripts. Manuscripts should be typewritten and double-spaced throughout, including tables, captions, references, etc. The title should be typed in capitals beginning at the normal lefthand margin of Page 1, and running across the entire page, with continuation on next line if necessary. Authors' names should be listed in all capitals below title, one author per line, with double-spacing between names. In footnotes please identify departmental source of article, and staff and academic ranks of all authors.

Abstract. A suitable summary, or abstract, should precede the article. The abstract should contain the conclusions of the article itself.

Figures should be drafted in black India ink, with legends typed on a separate sheet in numerical order, using Arabic numbers corresponding to figures. Glossy photographs are preferred. Medical drawings may be submitted in the original or in glossy prints. Photographs and figures should be lightly identified on back, as to number, top, and last name of author. Use Roman numerals to designate tables, and include descriptive headings under numbers. Indicate in text where figures and tables occur.

Abbreviations preferred are: ml, kg, gm, mg, μg, mm, and mEq/l.

References. Number references serially in text, and list in numerical order, beginning on a new page, as follows:

1. Gary GW, Bryan AC, Frayser R, Houston CS, Rennie IDB: Control of acute mountain sickness. Aerospace Med 42:81, 1971

List book references as follows:

2. Rafelson ME Jr, Brinkley SB, Hayashi JA: *Basic Biochemistry. Third Edition.* New York, Macmillan Company, 1971, pp. 96-208.

Abbreviations of journals should correspond to those in *Index Medicus.*

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RUSH-PRESBYTERIAN-ST. LUKE'S

MEDICAL BULLETIN

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VOL. 14, NO. 2 APRIL 1975

TABLE OF CONTENTS

PROTON (HEAVY ION) RADIOGRAPHIC DETECTION OF BREAST CARCINOMA AND OTHER PATHOLOGICAL STATES

V. William Steward

ABSTRACT. Radiations other than those of the electromagnetic spectrum or of sound may be used for diagnosis. These consist of beams of sub-atomic particles, particularly of protons and heavy ions. With these beams radiographs of unusually high contrast are obtained for the visualization and differentiation of the bodily tissues at very low doses. The method is practical for use in hospitals and has potential for screening purposes.

Nearly eighty years ago, on November 8th, 1895, Conrad Wilhelm Roentgen discovered "an agent [which] passes through a black cardboard envelope, which is opaque to the visible and ultra-violet rays of the sun or of the electric arc," and added, "we soon discover that all bodies are transparent to this agent though in very different degrees."[1] Thus, by working on a hunch, Roentgen caused x-radiography to be born, and within weeks it was being used in diagnosis. Certainly, few discoveries have had so immediate and enormous an impact; and now, after a span of eight decades, we see how sophisticated and invaluable x-radiography has become.

It is worth reflecting that our present knowledge of the physical world allows us essentially only three "windows" by which we may look upon it. Quantum mechanics based on the wave-particle duality of our fundamental entities suggests that categorizations may be somewhat arbitrary. Broadly speaking, there is first the "window" of the electromagnetic spectrum typified by light rays, x-rays, gamma rays, and infrared waves; the second "window" is more "mechanical" and is typified by sound and ultrasound. These two "windows" are in part used by our senses and are very extensively employed in various ways in medical diagnosis.

There is a third "window" which uses sub-atomic particles, particularly protons and other heavy ions. Somewhat surprisingly, these particles have not until now been used for medical diagnosis. In this lecture I would like to describe some of the properties of these heavy ions, in particular those of protons, and compare them with those of photons, e.g., x-rays. In this way I hope to show the considerable potential these particles have for providing a safe, non-invasive method for diagnosis, and I would like to illustrate this potential by the first results obtained in this area. Also, as the method is still in its infancy, some attempt will be made to predict what might be the developments of the future. For these reasons, I will not confine my remarks to breast cancer, but wish to broaden the discussion to a general consideration of the way in which proton and heavy ion radiography may detect pathological states in general.

THE PHYSICAL CHARACTERISTICS OF HEAVY IONS

A fundamental difference between photons, e.g., x-rays, and protons or other heavy particles is in the manner in which they interact with matter. In both instances, the interaction with the constituents of an absorber, e.g., tissue, is a statistical process. Photons can either lose their energy completely by a photoelectric in-

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Supported in part by NCI contract N01-CB-43918, B.A. No. N01-CG-3-2009, and by a donation from the Harry Rush Memorial Fund

teraction, or lose only part of their energy through Compton scattering. Thus photons are effectively removed from the beam when they interact with the tissue components. This removal is proportional to the incident intensity and results in an exponential type of attenuation curve. In contradistinction, the main interaction of protons and other heavy particles (except for neutrons) results only in a minute loss of the particle energy in any one event, so that there is little attenuation until the end of its range, where the energy loss becomes very great. In Fig. 1, these different types of attenuation are compared, and the very great difference in the radiographic contrast available between the two forms of radiation is shown.

Because of the statistical nature of these phenomena, the detection of lesions also is very much a matter of statistics. The kinetic energy of the particles is lost in discrete steps, mainly through interaction of the Coulomb field of the particle with those of the bound electrons in the absorber. This results in excitation and ionization of the absorber atoms, with ionization playing the major role. Because these interactions are statistically determined, a monoenergetic beam of particles passing through a homogeneous medium will show an energy spread and a near-Gaussian distribution (straggling) of stopping points around the mean total path length.

Relatively rarely, a nuclear interaction may occur which totally removes the particle from the beam. This accounts for the initial downward slope seen in Fig. 1 and, by influencing the slope at the end of the particle range, in turn influences the available contrast. More important, and again in contrast to x-rays, is the repeated deflection of the particle generally through small angles due to interaction with the Coulomb field of the electrons and nuclei of the target material. This interaction causes a spread of the particles in the plane of the detector and limits the spatial resolution capability when a direct recording device is used.

For a quantitative analysis of the energy loss incurred by protons as they travel through a medium, an equation derived by Bethe[2] is useful. This equation represents the energy loss of a proton by excitation and ionization along an element of path dx through the medium, the rate of loss being given by

$$\frac{-\,dE}{dx} = p\,\frac{Z}{A}\,\frac{2\pi N_0 z^2 e^4}{mv^2}\left[ln\,\frac{2mv^2W}{I^2(1-\beta^2)} - 2\beta^2 - 2\Sigma_i c_i - \triangle\right]$$

Here p, A, and Z are the density, atomic weight, and atomic number of the absorbing material; e and m are the charge and rest mass of the electron, respectively; N_0 is Avogadro's number, c is the velocity of light and $\beta = v/c$, where v is the proton velocity; z = effective charge of the proton; W = the maximum kinetic energy that can be transferred to an unbound stationary electron; I = mean exictation energy that can be transferred to the bound electron; $\Sigma_i c_i$ and $\triangle$ are the so-called shell and polarization correction terms. This formula applies to relativistic protons, i.e., those with a very high velocity and energy (above 2GeV). For diagnostic purposes protons of only 200 MeV (approximately) are required and for these the above formula may be simplified to;-

$$\frac{-\,dE}{dx} = \frac{4\pi e^4 z^2}{mv^2} N_0,\ B$$

where B (atomic stopping number) =

$$Z ln\left[\frac{2mv^2}{I}\right];$$

for 200 meV protons B = 0.5.

Energy loss thus depends directly on the density (p) and on the ratio Z/A of the medium. For most elements, particularly those of biological interest, Z/A is relatively constant, and it can be shown that the proton range depends on the atomic number Z roughly as $Z^{1/4}$ for elements from He to U. The exception is hydrogen, for which Z/A is twice as large; hence this element is very efficient in slowing down protons. Most materials with a

high hydrogen content have a lower density, however, and it is the density that is the predominant factor.[3]

Thus, if there is a mass of tissue with a density or hydrogen content which differs from its surroundings, those protons which pass through the inclusion will have a different energy loss than those that pass through the rest of the surrounding tissue. This is considerably different from x-rays, where the mass attenuation coefficient, μ/o, either varies as Z^4 for photoelectric absorption, or is independent of Z for Compton scattering. For x-rays in the diagnostic energy range, photoelectric absorption and Compton absorption are equally important, and thus attenuation varies approximately as Z^2.

Also, from the Bethe equation it can be appreciated that $\frac{dE}{dx}$ increases as the particle slows down. This results in a large maximum known as the Bragg peak, located toward the end of the particle range. This situation is very different from the exponential dose distribution seen with x-rays (or with neutrons). In Fig. 2 these different depth-dose distributions are compared. The figure shows where a detector, e.g., a screen-film combination, may be placed. Importantly, it also illustrates that the Bragg peak of ionization may be positioned well beyond the region being examined so that the dose delivered throughout the depth of the tissue approximates that at the first surface.

The detectability of areal density changes from one image element to another is determined in part by the precision with which the mean range of the particles forming it can be measured. This precision is affected by the straggling of the particles which, in turn, is influenced by their mass. A detailed comparison has been made for a number of ions under ideal conditions concerning their ability to pick out a $1mm^2$ picture element (of areal density changes $(\triangle 1p) = 0.3\%$) in a biologically simulated situation.[4] The results show that, although the necessary fluence is less with the heavier ions, as expected, because of their lesser straggling, the dose required (except for the ions of deuterium and tritium) is higher, by about a factor of two, than for protons. More importantly, the energy and "rigidity" of the heavier ion beams is considerably greater than that for protons and makes the accelerators necessary for their production very much more expensive. This has very significant practical applications, as we will see later.

From the attenuation curves (Fig. 1), it can readily be appreciated that, for a given input, a larger fraction of protons than photons can penetrate an absorber. More particles survive to tell their tale, so to speak, and thus a great deal more useful information is available at the detector level from a beam of heavy ions than from a beam of x-rays. This is a crucial difference between the two forms of radiation.

Also it is of interest that from the mechanisms described earlier we can broadly say that the images produced by x-rays are largely formed by those photons which do not interact with the tissue components, whereas with protons most of the particles help with image formation. It must be remembered, however, that all of the information is impressed on the proton or heavy ion beam as soon as it has passed through a density anomaly. Thereafter, the effects of scattering and straggling combine to degrade the information as it passes through additional absorber. For this reason, it is important that proper consideration be given to minimizing the total amount of absorber interposed in the particle beam (thus, by implication, to matching the beam energy to the sample), and to the proper placement of detectors. For example, with a uniform beam and a down-stream detector, e.g., film, a lesion is best visualized when it is near the film. Conversely, during scanning with a collimated beam without position resolution in the detector, the image is best detected when it is located near the front surface, i.e., before degradation of the beam has occurred.

The multiple Coulomb scattering mentioned above can be calculated. The root mean square value (y rms) of the dis-

place of the particle at the end of its range perpendicular to the initial direction of motion is given by[5]

$$\frac{y \text{ rms}}{R} = 0.3 \left[\frac{Z}{E_0}\right]^{1/2} W^{0.1}$$

where R = range of the particle,
Z = atomic number of the absorber, and
W = proton kinetic energy in units of E_0.

The effect is rather like working with a large focal spot and limits the spatial resolution capability of the end-of-range method. Nevertheless, advantage can be taken of this mechanism to produce radiographs analogous to those obtained in xeroradiography. These outline the edges of internal structures and produce better spatial resolution. In fact, scatter proton radiographs of a mouse compare very favorably with the corresponding conventional x-radiographs.[6] Also, it can be seen from the equation that the scattering varies as $Z^{1/2}$, compared with $Z^{1/4}$ for the end-of-range technique. However, scatter radiography is not as sensitive to density variations as the marginal-range method, and would not be of much use for the detection of anomalies with ill-defined edges. Thus, it is unlikely that the method will play much of a role in diagnosis.

Some idea of the sensitivity obtainable with protons may be apparent from the fact that investigations with film and monoenergetic proton beams have already demonstrated a capacity to image thickness changes as small as 0.05 percent in idealized cases. Also, with a split-beam scanning method where one part of the beam acts as a reference, extremely high detection of the order of 50 ppm in effective absorber thickness ($l\triangle p$; areal density) has been obtained.[7] These are far smaller than those observable with other radiographic techniques. Even in practical cases, changes of 0.2 to 0.5 percent are not only achievable, but would appear adequate for most purposes.

EXPERIMENTS TO DATE

The first experiments on proton radiography of human tissues were performed on fixed autopsy material, with the 160 MeV proton beam from the Harvard cyclotron.[3,8-10] The beam from this accelerator is 0.5cm in diameter. For uniform illumination of the specimen, the protons were transmitted through a lead or copper scatterer. The specimen was placed about 3 meters from the scatterer and was immersed in water or other tissue-equivalent fluid contained in a plastic box with parallel faces. By this means, the effects of shape variation were minimized and the density variations within the specimen highlighted. Photographic film, usually Polaroid TLX film, was then placed immediately against the exit face of the water box, in the region of the far downward slope of the Bragg peak. Fig. 3 shows the setup employed, including the thin polystyrene absorbers used to adjust the incident beam energy so that radiographs of optimum contrast were obtained.

Figs. 4 and 5 illustrate the first pictures taken with this method. In controdistinction to the x-radiographic method, both primary and secondary human brain tumors are well visualized. In addition, the ventricular system is well outlined and the basal ganglia are seen.

Fig. 6 demonstrates that it is possible to record directly a non-calcified brain tumor inside a skull despite the considerable modulation of signal due to the overlying cranial bones. This result is of some interest since it simulates the clinical situation and, as far as we are aware, represents the first time that a non-calcified brain tumor has been recorded directly on film.

Other cerebral pathological conditions may be visualized as well. Figs. 7 and 8, respectively, indicate the potential of the method in directly revealing intracranial hemorrhages and cerebral infarctions. The lesions of multiple sclerosis have also been visualized.

Our attention has not been confined to the brain, however, but has included a study of breast carcinoma. This was undertaken for a twofold reason: first, because the breast is an external organ and readily lends itself to investigations of this

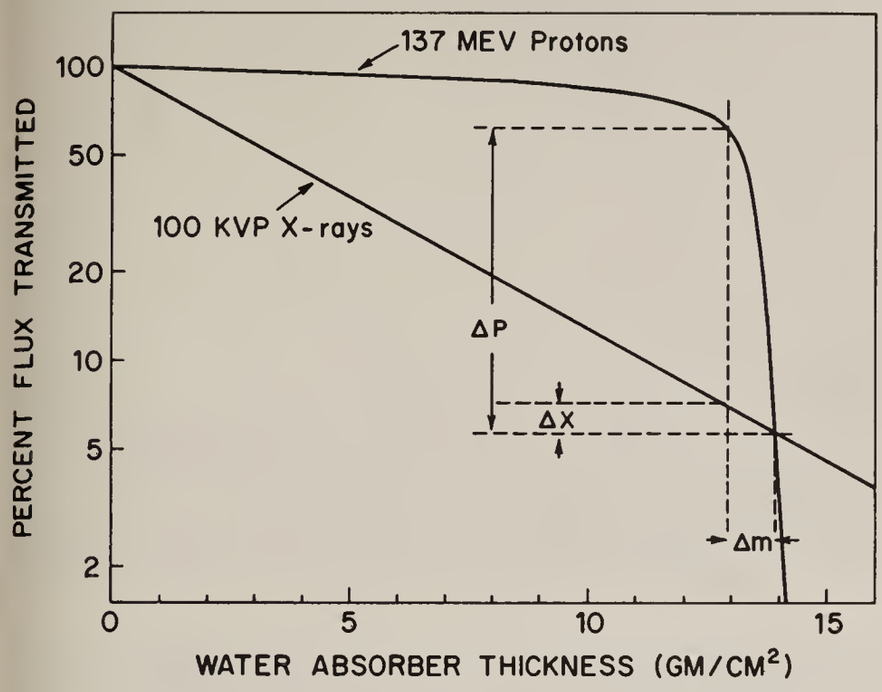

Fig. 1 — Flux-depth curves for protons and x-rays passing through a homogenous medium. Note the difference in available contrast between the two rays for detection of mass difference.

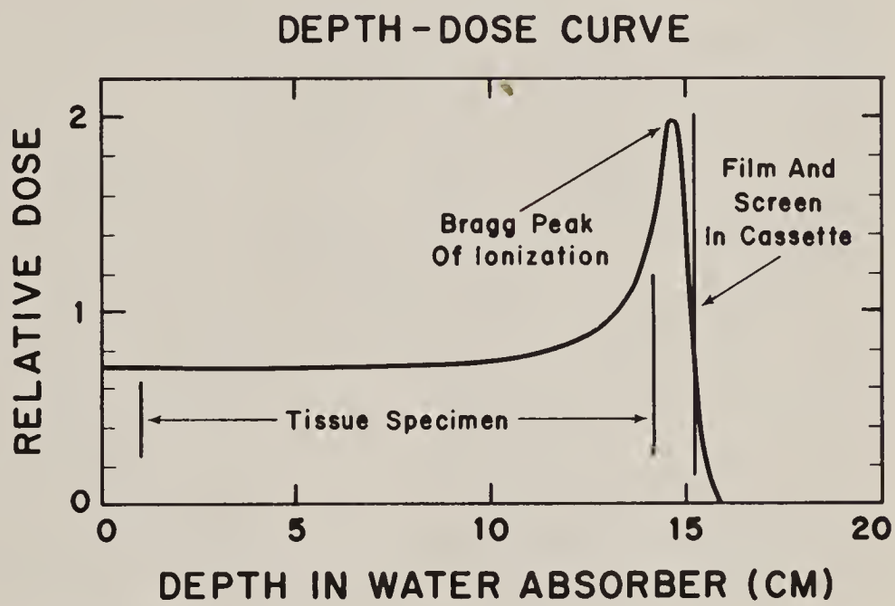

Fig. 2 — Dose/depth curve for 148MeV protons in water, showing the Bragg peak of ionization near the end of the particle range. The position of the tissue specimen and of the film and intensifying screen relative to the Bragg peak are indicated.

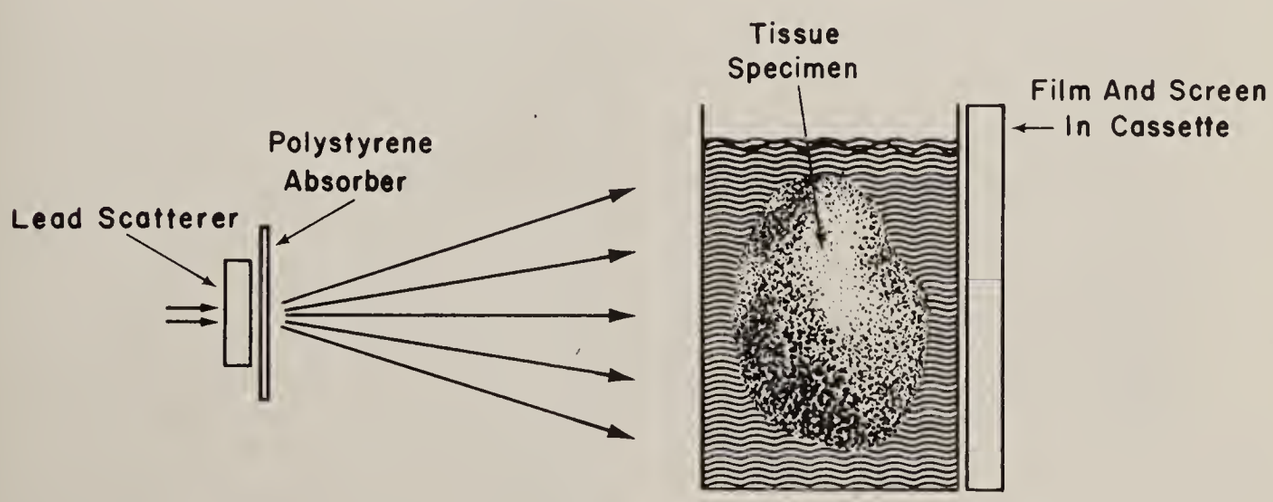

Fig. 3—Demonstration of the experimental setup for study of specimens.

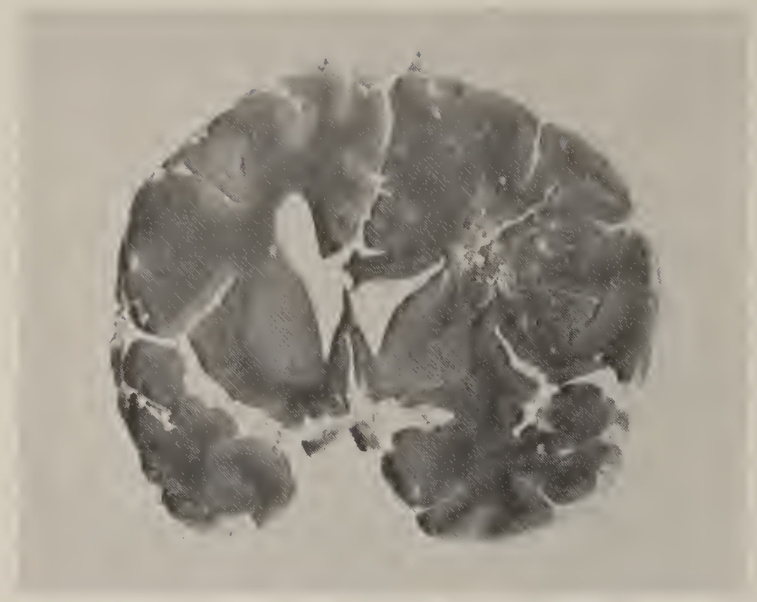

Fig. 4a—Front view of a coronal slice 1 cm thick of a human brain fixed in formalin. Note the tumor, a glioblastoma multiforme, in the white matter of the hemisphere on the right, with swelling of the hemisphere and distortion of the ventricular system.

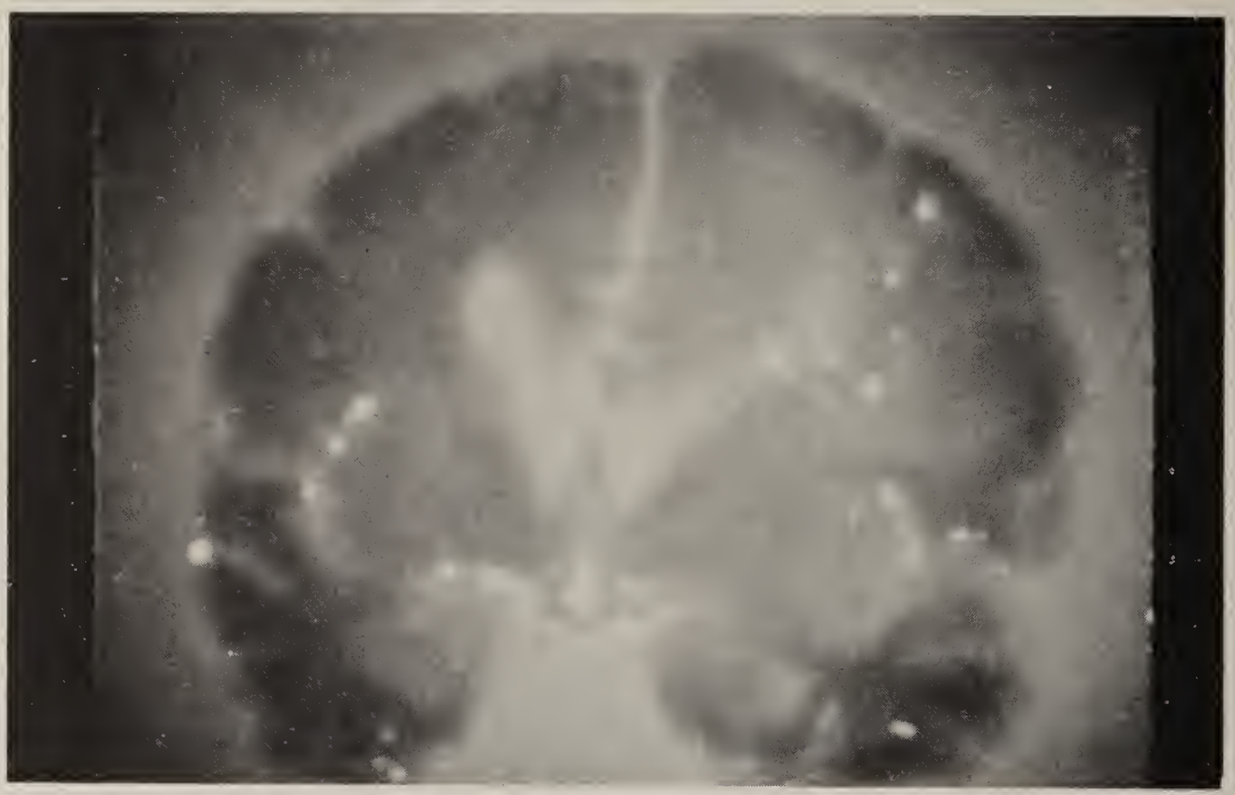

Fig. 4b—Proton radiograph (positive). Note decreased density in the tumor area and visualization of the basal ganglia. White spots are air bubbles. The conditions were 115 cm focus to film, no screen, Polaroid TLX film, and a dose to the first surface of approximately 290 rad in 0.8 minute.

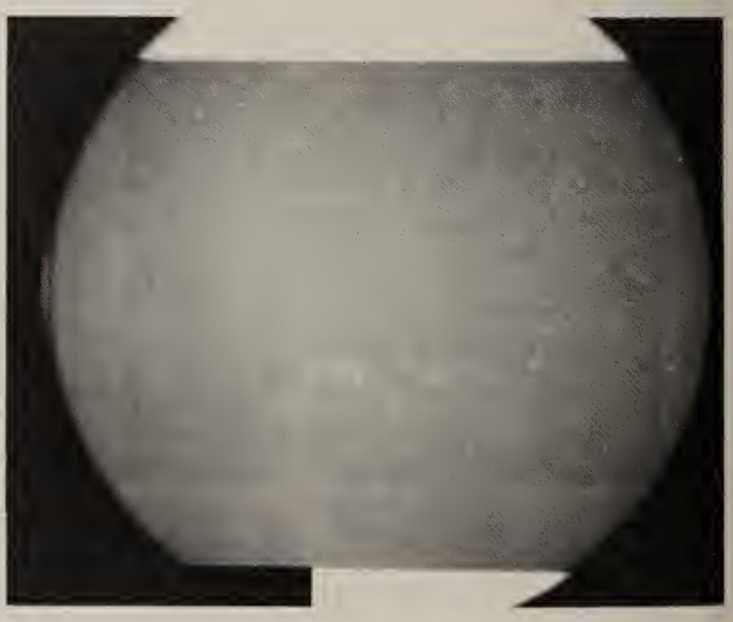

Fig. 4c—Optimal x-radiograph of brain slice in water tank. (Note that air-bubbles are visualized.)

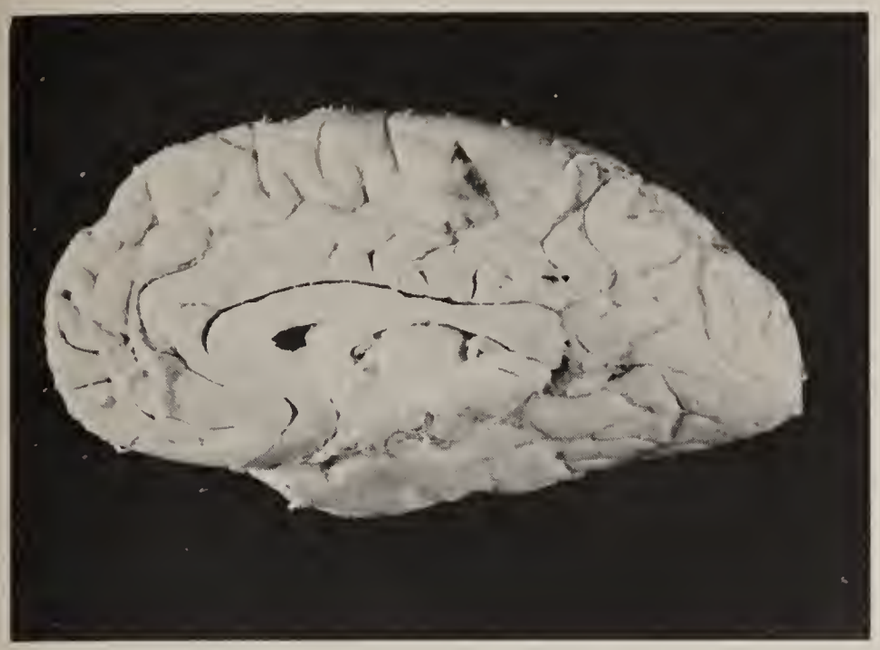

Fig. 5a—Medial view of formalin-fixed right hemisphere,, showing metastasis from a mucin-producing adrenocarcinoma of the pancreas below the midpoint of the superior border.

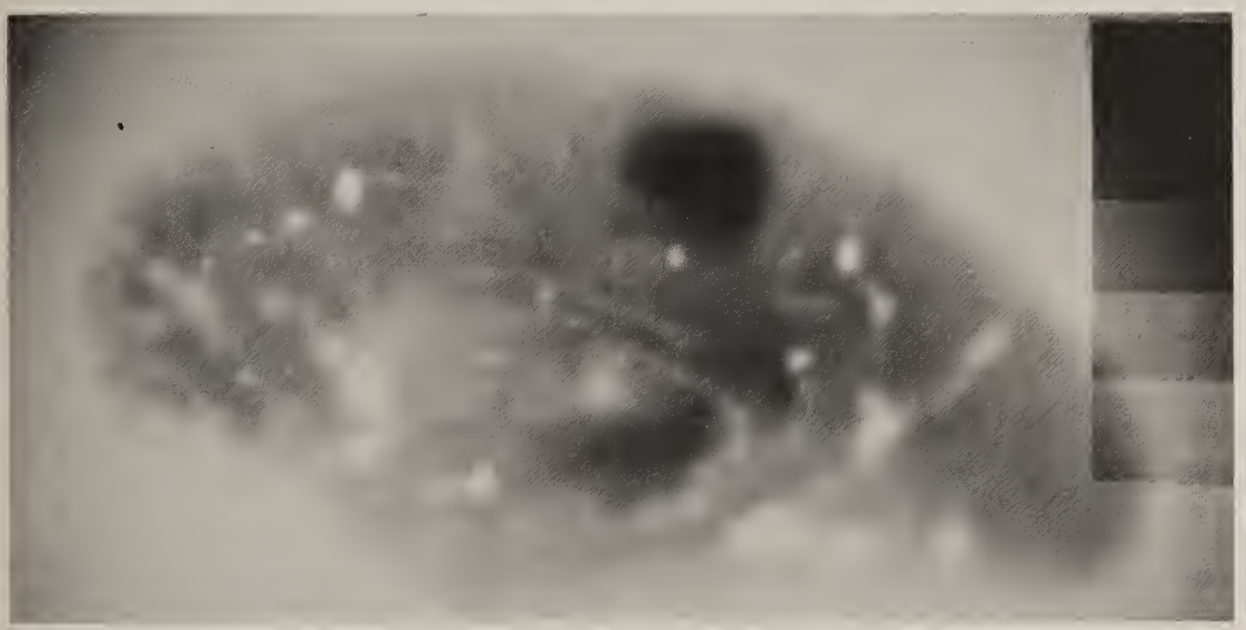

Fig. 5b—Proton radiograph of the hemisphere (positive). The conditions were 115 cm focus to film, no screen, Polaroid TLX film, and a dose to the first surface of approximately 290 rad in 0.8 minute.

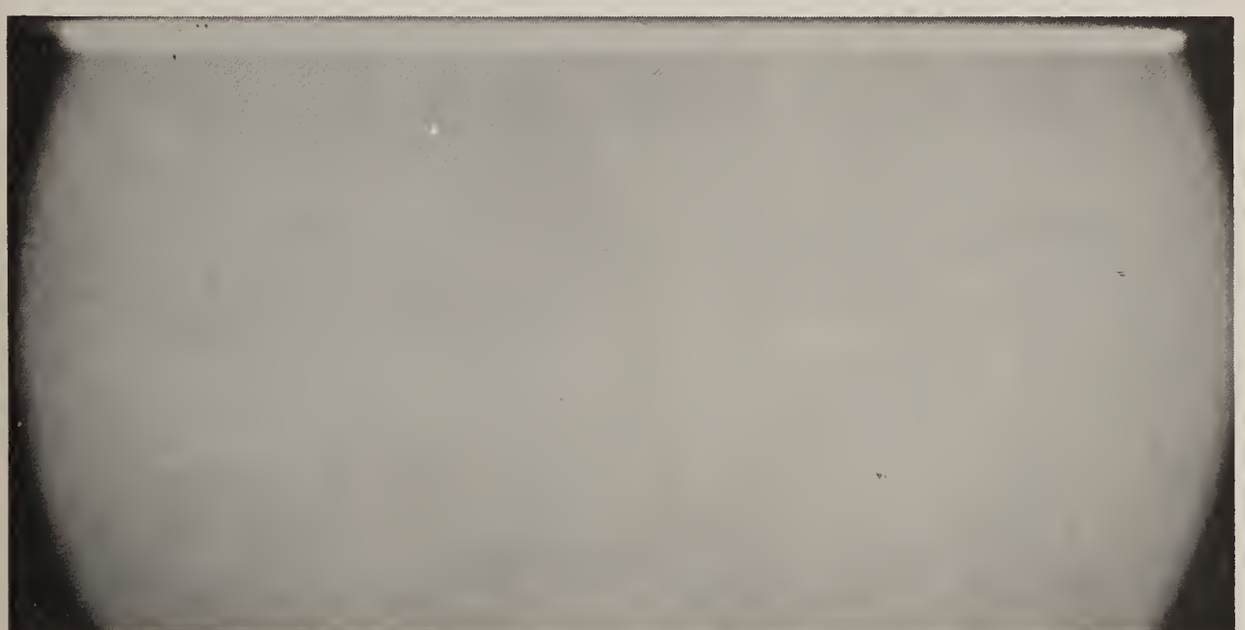

Fig. 5c—X-radiograph of the hemisphere in a plastic water box. The conditions were 126 cm focus to film, Du Pont high-speed 1A screen, Polaroid TLX film in Polaroid cassette, 30 KV peak, 200 MA, 3.0 seconds.

Fig. 5d—X-radiograph of the hemisphere in air. The specimen was reconstructed from serial coronal sections. Note that the tumor is just visible. The conditions (optimal) were 92 cm focus to Kodak mammography film, 27 KV constant potential, 20 MA, 2 minutes.

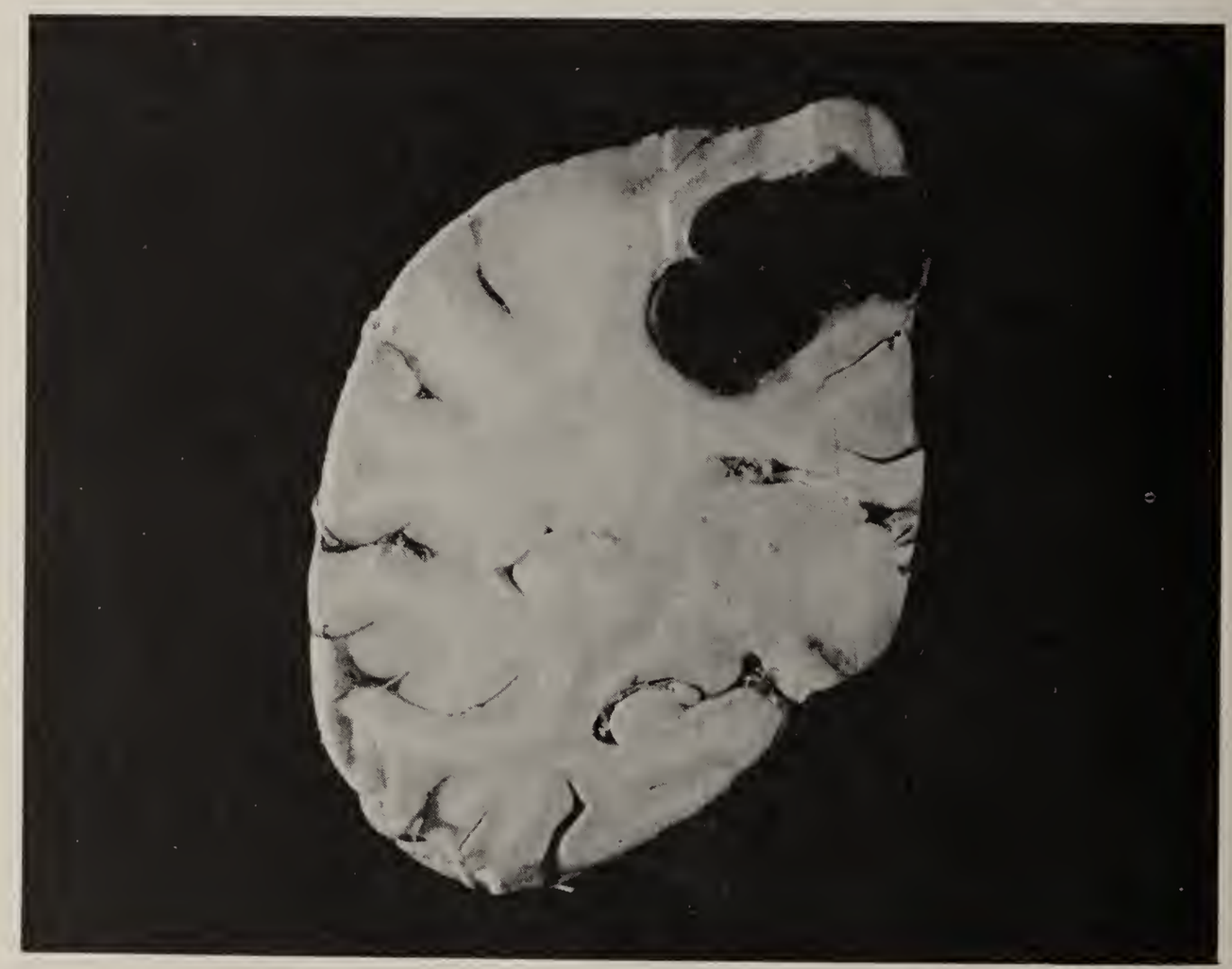

Fig. 5e—Photograph of a slice taken through the tumor.

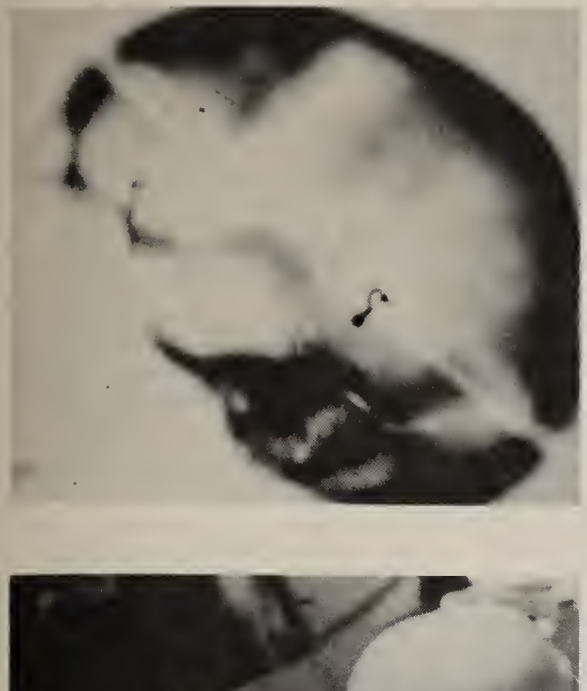

Fig. 6a—Proton radiograph (positive) of normal formalin-fixed human brain autopsy specimen placed within a skull in a water tank. Comparison with a similar picture taken with the brain removed (not shown) indicates some areas of increased density coming in from the periphery. Polaroid TLX film. Radelin TF intensifying screen. First surface dose less than 0.5 rad.

Fig. 6b—Photograph of the right hemisphere of a human brain (specimen in Fig. 5) placed within the lower portion of a skull. Two strips of lead attached to the brain point towards a small portion of the tumor extending to the medial surface of the hemisphere.

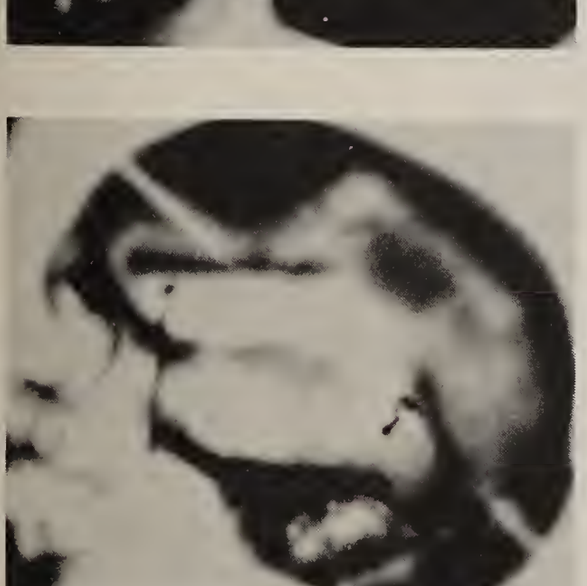

Fig. 6c—Proton radiograph (positive) of the tumor-bearing hemisphere held in the lower portion of the skull as illustrated in Fig. 6b. Note ends of the lead markers pointing to the lesion. Polaroid TLX film. Radelin TF intensifying screen. First surface dose less than 0.5 rad.

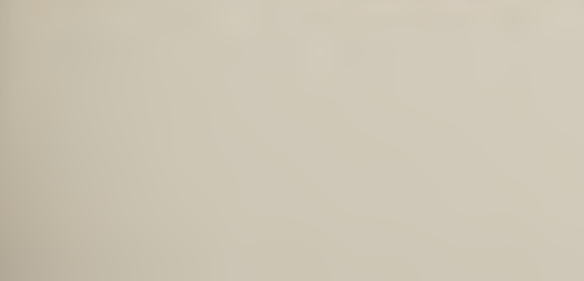

Fig. 6d—Proton radiograph (positive) of the brain and tumor in the complete skull. With the skull cap in place, despite modulation of density due to variable bone thickness, the tumor density can still be seen. Polaroid film. Radelin TF intensifying screen. First surface dose less than 0.5 rad.

FIG. 7—INTRACEREBRAL HEMORRHAGE.

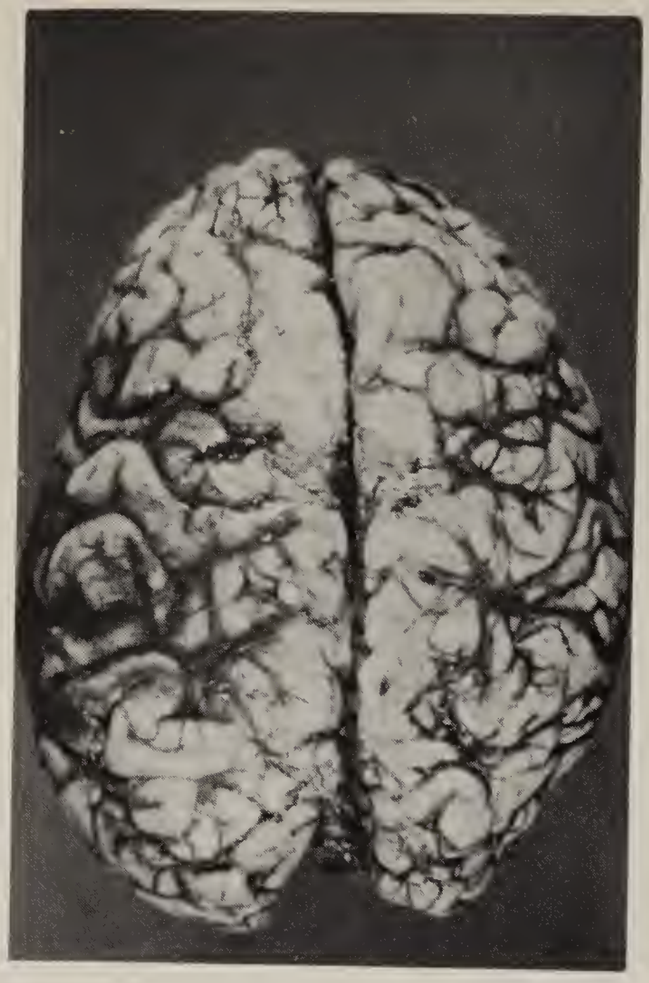

Fig. 7a—(Left) Photograph of superior view of brain. Note slight discoloration and swelling of left hemisphere which contains the lesion.

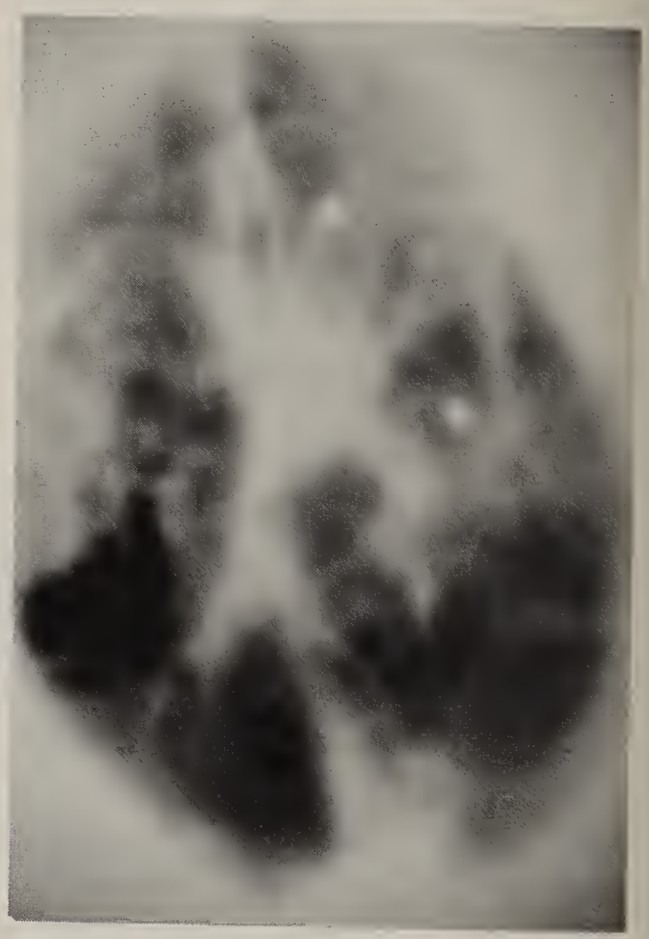

Fig. 7b—(Right) Proton radiograph (positive) taken through vertical thickness of specimen. Note dense area on left corresponding to intra-cerebral blood clot.

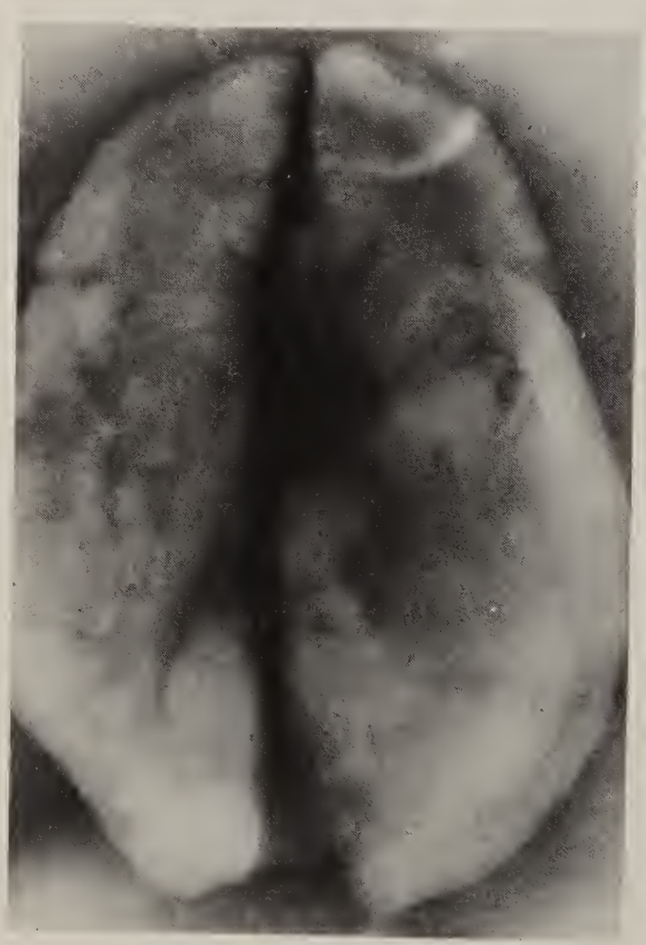

Fig. 7c—(Left) X-radiograph through vertical thickness of specimen in air using 2 MeV Van de Graff electrostatic accelerator.

Fig. 7d—Proton radiograph (positive) of brain viewed transversely.

Fig. 7e—Proton radiograph (positive) of brain viewed transversely with specimen immersed in calcium chloride solution the density of which approximates that of the brain (specific gravity 1.038).

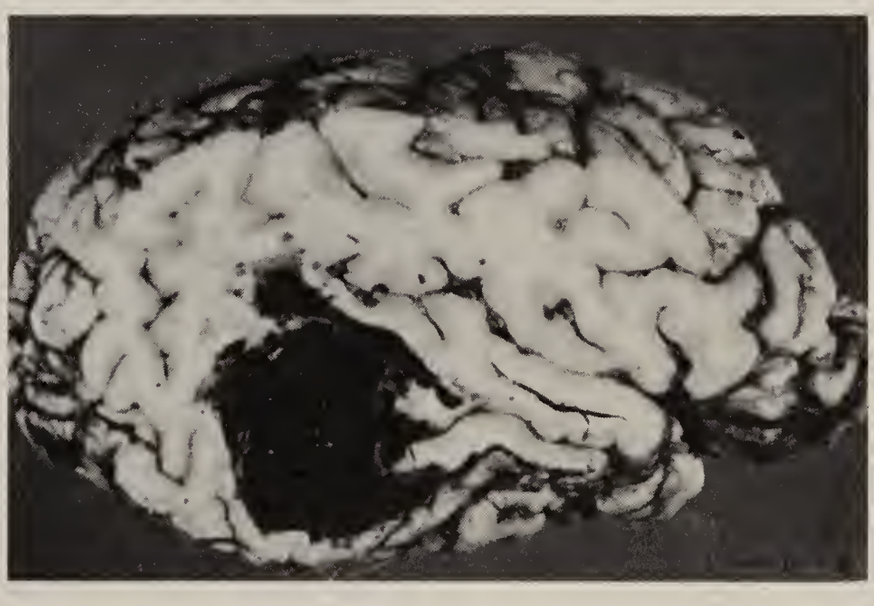

Fig. 7f—Photograph of side view of brain showing blood clot.

FIG. 8—CEREBRAL INFARCTION.

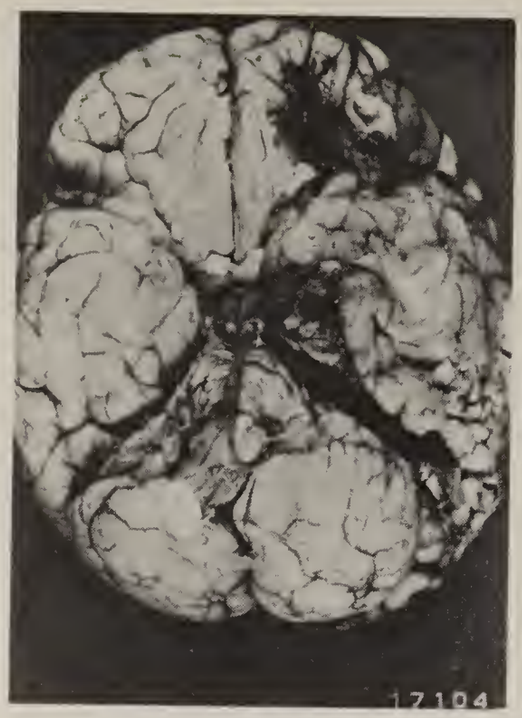

Fig. 8a—Photograph of inferior view of brain with massive infarction of left hemisphere (on right in picture) due to recent thrombotic occlusion of the left middle cerebral artery.

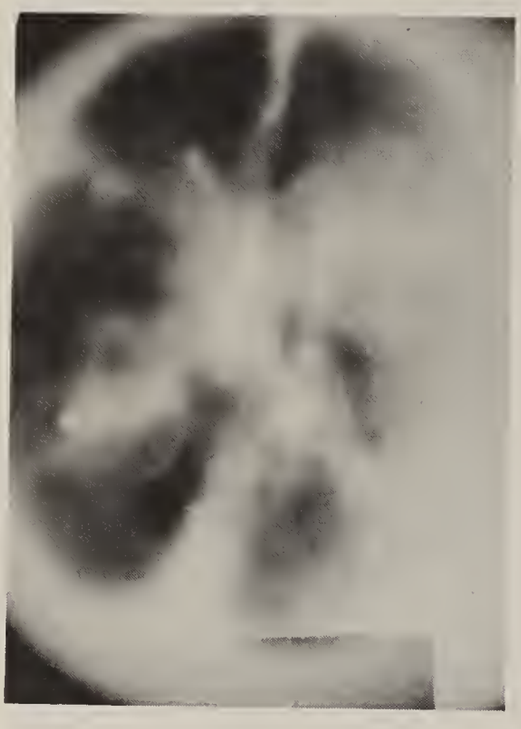

Fig. 8b—Proton radiograph (positive) of brain. Note less dense left hemisphere (on left in picture). Dense features towards lower right is the medulla and upper cervical cord.

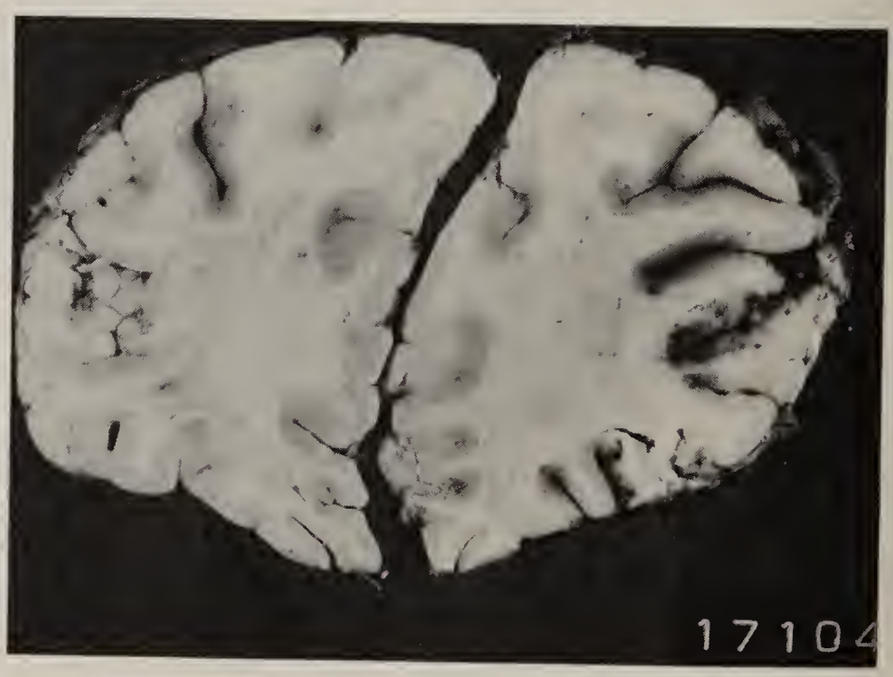

Fig. 8c—Photograph of coronal section through frontal lobes of brain with infarcted hemisphere on right.

kind, and secondly, because of the pressing need for a safe diagnostic method for detecting "early" lesions. Fig. 9 illustrates our results with fresh radical mastectomy specimens; Fig. 10 represents an extension of these studies and illustrates that the findings on fresh specimens are applicable to the living patient. Of particular interest was the revealing of small satellite tumors not previously suspected in two of the cases.

The work on fresh human specimens was continued in early 1974 with the facilities of the High Energy Physics Division at the nearby Argonne National Laboratory. Here fresh breast and brain specimens were moved on an automatic X, Y stage in front of a 0.25" diameter beam of 200 MeV protons. The percentage flux transmitted per ¼" x ¼" picture element was recorded and "isodensity" contours drawn. By this quantitative scanning method, a fresh intracerebral hemorrhage was clearly and correctly located in a hemisphere, as was a cancer in a fresh mastectomy specimen.

Apart from bearing out the theoretical prediction that soft tissues should be visualized better by heavy ions than with x-rays, an important aspect of this work has been the low radiation doses employed. Thus, the carcinoma in the breast of the living patient was revealed with a first-surface dose of slightly under 0.3 rad (conventional x-mammography: 6-15 rad); with the scanning method on the mastectomy specimen, 20,000 protons were incident on each picture element, giving a first surface dose of approximately 3.4 millirad. Similarly, the fresh brain specimen was scanned with 10,000 protons per picture element, giving a surface dose of only 1.7 millirad. Considering the good statistics, these doses could have been considerably reduced without noticeable detriment to the formation of a diagnostically useful image. In fact, with an electronic detector a recognizable image of a biological test object has been obtained with an incident dose of about 1×10^{-4} rad, equivalent to the radiation one experiences in 8 hours' existence on the surface of the earth.[11]

These facts suggest that very low doses of heavy ions will give diagnostically useful images, although the actual level will obviously depend on the amount of information required. However, the doses required suggest that we have a lot of latitude in this matter before reaching anything like the doses encountered with x-rays. Furthermore, a number of studies, done with a wide variety of biological test systems, indicate that the relative biological effect of protons is slightly less than that of x-rays (RBE = 0.8).[12,13] This further enhances the safety of the method.

Most recently, several other important developments have taken place in this field, one being the fabrication at Argonne National Laboratory of a more sophisticated scanning system than that previously used. This latter system utilizes a 50-foot-long collimator unit from which an array of four square 200 MeV proton beams emerges, each 1mm on a side. These beams pass through their individual up- and down-stream scintillation counters which are placed on either side of a water box containing the specimen. Scanning is accomplished by moving the specimen at 1mm intervals. Measurements are derived from the ratio of the readings from the two sets of counters and are stored on magnetic tape from which the data can subsequently be displayed. With this system, a series of measurements has been made on a variety of physical test objects, and certain theoretical predictions confirmed by experiment. This has given us a better foundation for understanding some of the physical aspects of proton radiography. Later we hope to use it to investigate a wide variety of pathologic specimens.

Another development, which is well advanced, and which is crucial for a proper understanding of the potential of heavy ion radiography, is an investigation into the densities of fresh human tissues. Surprisingly, a search of the literature going back over 100 years shows this to be a subject that has been inadequately studied, particularly with regard to the soft

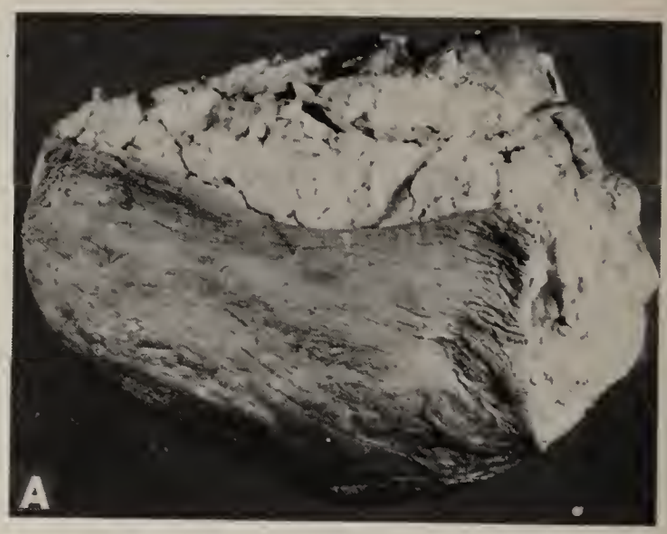

Fig. 9a—Case I. Radical mastectomy specimen seen in side view.

Fig. 9b—Proton radiography I (first surface dose, 0.3 rad). Side view of the specimen in the water tank, oriented as in Fig. 9a. Floating paraffin blocks are seen above the specimen. Note the wedge-shaped densities on both sides, representing portions of the pectoral muscles, and the irregular dense area on the right. Polaroid TLX film and a DuPont HS1A screen were used.

Fig. 9c—Proton radiograph II (first surface dose, 0.9 rad). Same view as in Fig. 9b, but taken with a longer exposure. A plastic probe was inserted into the biopsy site. Note the increased size and density of the areas seen previously and the small dense area on the left.

Fig. 9d—Section through surgical specimen shows a firm, irregular mass surrounding the biopsy site (middle arrow) and extending to the right (right arrow). The involved region corresponds to the irregular dense areas seen on the proton radiographs. Note the small triangular tumor area to the left (left arrow), corresponding to the small dense area described previously.

Fig. 10a—Case II. Radical mastectomy specimen, showing the site from which tissue was taken for biopsy.

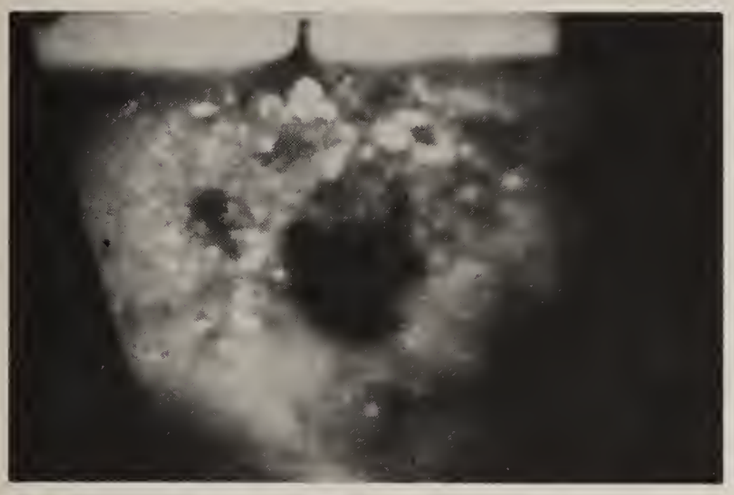

Fig. 10b—Proton radiograph (first surface dose, 0.6 rad). Front view of the specimen, with a plastic probe protruding obliquely from the biopsy site (center). Note the smaller dense area to the left. Polaroid TLX film and a Du Pont HS screen were used.

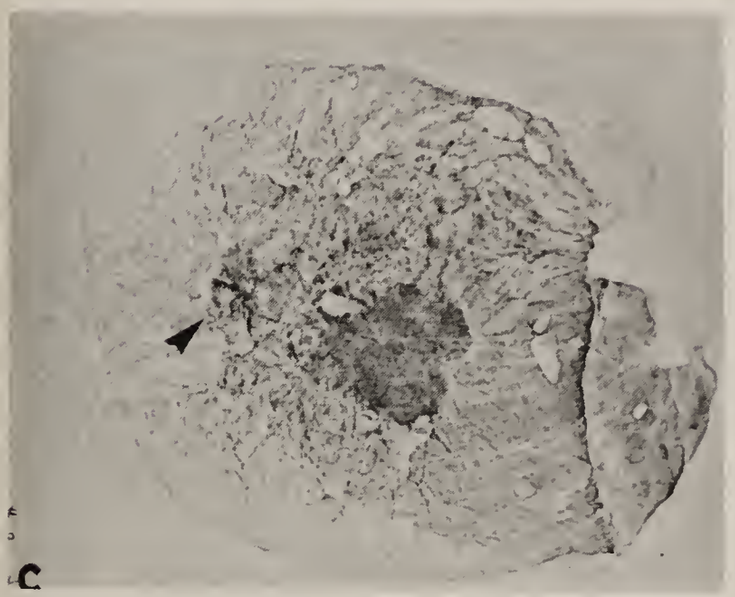

Fig. 10c — Xeroradiograph of the same specimen in air, taken at 35.5kV, 200mA, 2 seconds, at a dose of 1,800mR and a tube-film distance of 0.91 m (36 in.). The arrow indicates the satellite carcinoma.

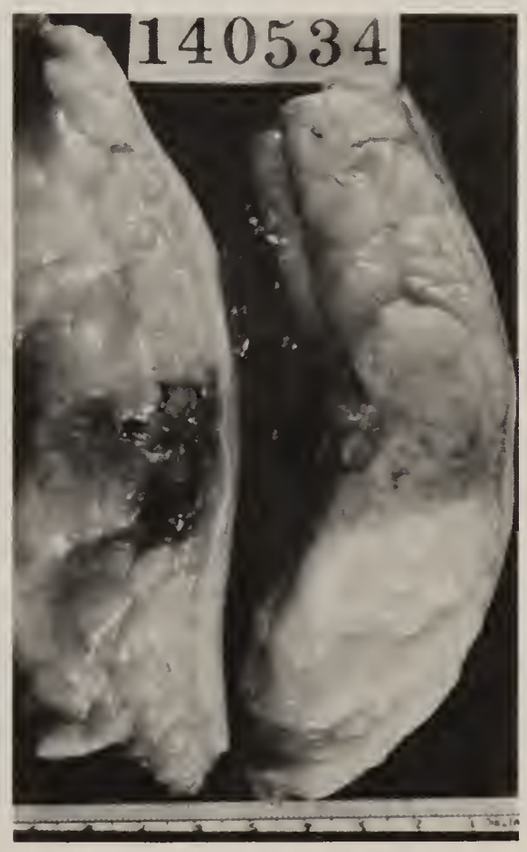

Fig. 10d—Sections through the specimen revealed a firm mass approximately 7cm in maximum diameter, with the outer margin extending 4cm from the closest margin of resection. A second smaller mass was noted in the region corresponding to the small area of increased density seen in the proton radiograph.

Fig. 11a—General view of the experimental set-up for studies of living patients (collimator to left). The beam was carefully adjusted to irradiate only the water box, into which the breast protruded. A Polaroid TLX film cassette (used with a DuPont HS screen) was placed against the far side of the water box.

Fig. 11b—Close-up view of the breast in the water tank. Note the fungating mass on the left.

Fig. 11c—Proton radiograph (first surface dose, slightly less than 0.3 rad). Note the dense area on the left, corresponding to the site of the tumor and its extension downwards and to the right.

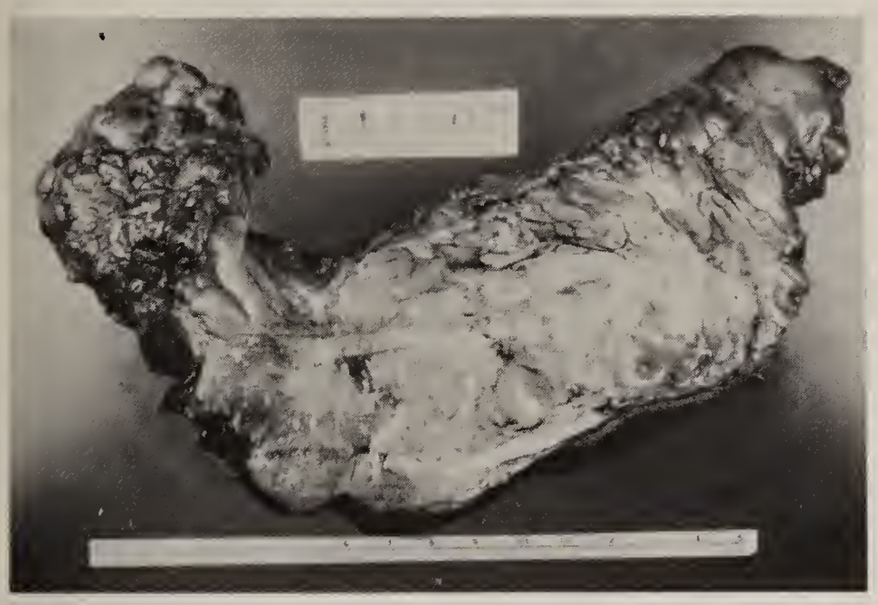

Fig. 11d — Section through the surgical specimen. The tumor mass occupies the region to left and extends downwards through the skin.

body tissues, from the radiographic point of view. In order to obtain some data, we have set up a series of density gradient columns which are sensitive to 10^{-5} g/cc. To be of value, such an investigation is highly dependent on the cooperation one receives from one's colleagues regarding the specimens, and here I would like to express our very great appreciation to all the members of your pathology department, in particular Doctors Hass and Danauskas, for their tremendous cooperation in this matter. With their help we have been able to look at every organ of the human body and at a wide spectrum of pathologic conditions, with results which have been most interesting. It would take up too much space to detail our findings, and besides, the investigations are still in progress; but we can say in general that the solid organs and their subdivisions do possess characteristic density differentials and that neoplasms, whether primary or secondary, likewise possess densities which differ from their surrounding. Most importantly, these density differences are highly significant when considered in relation to the sensitivity of the proton and heavy ion radiographic method, and they give us hope that very small lesions may eventually be detected with very reasonable radiation doses to the body.

POTENTIAL APPLICATIONS OF PROTON AND HEAVY ION RADIOGRAPHY

The question that may now legitimately be asked is, "How useful will proton and heavy ion radiography be in medical diagnosis?" This obviously is a complex question, and although a specific answer cannot be given at present, there are many indications suggesting that its application should be considerable. As will become apparent, much of this usefulness stems from a very fortunate combination of a wide variety of dissimilar factors.

Fundamentally, the technique can be said to open up a new "window" for viewing the internal structure of the body. It utilizes tissue parameters which have not been exploited by any previous diagnostic method. On this basis alone it should have potential. Furthermore, there is the very considerable body of knowledge accumulated by the physicists, giving us as much knowledge of the interaction of heavy ions with matter as we have for x-rays. This has provided us with a firm foundation from which we may pursue our work. Also, the tissue characteristics which determine the penetrability of heavy ions can be measured easily and accurately. In this respect, heavy-ion radiography stands in sharp contrast to techniques such as the brain scan which depends on penetration of the blood-brain barrier by radio-isotopes; as yet, little is known of barrier dysfunction, however.

It is fortunate that the energies required for penetration of the body are near 200 MeV. At higher energies, say, 300 MeV or more, nuclear interactions become increasingly important and attenuation more nearly exponential, and, as with x-rays, with resultant loss of contrast. Yet another advantage lies in the fact that, although higher energies may be required for penetration through such regions as the pelvis, in contrast to x-rays, this will result not in a higher, but in a lower delivered dose. The effect is slight, but nonetheless in the right direction, and is important when genetic effects and the safety of the fetus *in utero* are considered. Furthermore, and on a somewhat different level, proton radiography has the additional advantage that beams of monoenergetic particles are not difficult to produce, and that, from the viewpoint of technology, accelerators for hospital use are a practical proposition.

Chief usefulness of the method resides in its capacity to visualize soft bodily tissues and to differentiate one from another, including pathologic states, in many ways much better than x-rays. Certainly this is true when we compare equal doses of protons, or other heavy ions, and photons. It may be argued that, in general terms, x-rays are most useful in showing up the bony and hard tissue components of the human body. We recognize, however, that most of the body, and most of the diseases which afflict it, involve soft-tissue components, and that it is only by special contrast studies that some of these are visualized by x-rays. Even then, many abnormalities are made apparent only indirectly. With heavy ions, the reverse appears more true; one hope is that fewer contrast studies will be necessary, and that, indeed, in many instances, none may be required.

Based on the great difference in available contrast between protons and photons, I would further venture the guess that there will be many situations in which lesions will be detected by heavy-ion radiography, but not by x-radiography. This is suggested in a very preliminary sense by our present results, and is advanced despite the recent introduction of such sophisticated pieces of equipment as the EMI and ACTA x-ray scanners. For although (or perhaps because) these marvelous devices provide very efficient photon detection, they are nonetheless limited by the intrinsic nature of x-rays, and therefore multiple probings are still required for imaging of the soft tissues. Not only does this impose a concomitant increase in dose to obtain the increased information, but it also requires that the spatial resolution be dependent more on computer reconstruction techniques and

less on the qualities of x-rays which *per se* can give very good spatial resolution.* Furthermore, as they presently stand, the EMI and ACTA scanners look at cranial slices that are 7 to 8mm thick. Personal experience in cutting many hundreds of human brains has taught me that the brain anatomy can present surprisingly different aspects at levels separated by such distances, and integrating over these depths could "wash out" a number of interesting details. This is not to say that the results obtained by computerized tomography are not of the highest possible interest, but by being able to reconstruct slices 1 mm thick, one may discover further features of diagnostic value. With doses comparable to those now used by the EMI scanner, this would be possible with heavy-ion radiography. But, and this is an important consideration, heavy-ion radiography may often make two-dimensional reconstruction methods unnecessary. It is conceivable that two views at right angles to one another, or at angles suitable for stereoscopic viewing, would be sufficient to detect and locate a lesion accurately enough for both the surgeon and the radiation therapist. Also, as a corollary, the method would pinpoint lesions with the accuracy required by the various types of nuclear therapy now coming into vogue. One can envisage the initial detection and localization of a lesion by heavy-ion radiography and subsequently, by the push of a button, the selective irradiation of the required region. This may not be too far off, for even now several groups of physicists are already addressing themselves to the design of accelerators which might perform this dual function.

More specifically, we may ask, "In what areas may this new form of radiography help in diagnosis?" Already we have presented evidence suggesting its capability for detecting a wide variety of intracranial lesions, including demyelinating conditions. As with so many other parts of the human body, however, a given tissue can react in only a few prescribed ways to a host of injuries. Secondary effects may occur, and for a precise diagnosis, we need not only the site and size of a lesion, but as much information on the fine modulations which occur both within and beyond its boundaries. For effective therapy, another requisite is that lesions be detected at the earliest possible stage.

Essentially, it would appear that heavy-ion radiography could detect most of the macroscopic lesions of the brain, including cerebral edema. Likewise, it appears capable of detecting breast neoplasms, and very importantly, because of the low doses involved, could be safe for screening of patients at risk. Such screening could also be applied to many other conditions such as the granulomatous and cancerous lesions of the lung.

An important consideration in any discussion concerning the potential of this new method is that the human body, composed as it is of gas, liquids, and solids, presents an enormous range of densities for analysis by heavy-ion radiography. At the lowest end is air with a density of 0.0012, followed by liquids of known density such as blood (S.G. 1.055), urine (S.G. 1.007-1.030), and cerebrospinal fluid (S.G. 1.005-1.009). At the other end of the scale are the solids, for which our measurements show fatty tissue to have a specific gravity of about 0.92, and the non-fatty solid organs where the range is from 1.02 to 1.07. When one considers the juxtaposition of widely disparate densities within the living body, these findings, combined with the results obtained for a variety of pathologic conditions, should make it possible, as mentioned earlier, to visualize a host of disease states. The list would include virtually the whole of the human body. Thus, the method could be applied to the detection of both primary and secondary neoplasms in the majority of organs, hopefully distinguishing benign from malignant tumors, and the differentiation of these from non-neoplastic conditions.

*Recent work on the linear attenuation coefficients (μ) of tissues for gamma rays suggests that the EMI scanner may depend on variations in blood supply for visualizing lesions, rather than on μ or variations in tissue atomic weight.[14]

For example, diseases of the lung, whether primary or secondary, focal or diffuse, could be detected. Because this organ approximates an air-filled sac, fluid accumulation in the parenchyma as occurs in pulmonary congestion, edema, or infarction, should be easily visible, as would a variety of pleural effusions. For the same reason, focal lesions smaller than those detectable elsewhere should be seen because of the enhanced contrast. Diffuse lesions such as those produced in silicosis likewise should be detectable. In these cases, early lesions may be too small to be seen individually; but because they add mass to the organ, the particle range would be reduced. Also, the random distribution of the lesions would tend to widen the Gaussian distribution of the stopping points of the particles compared with the normal distribution. With proper design of detectors, both features could be visualized.

Density measurements indicate that myocardial infarction also could be imaged with appropriate instrumentation, probably by two-dimensional reconstruction methods. Certainly the macroscopically visible lesions of infarction are significantly different in density from the surrounding myocardium, but it remains to be shown that the all-important acute ischemic state and its gradations can be adequately visualized *in vivo*. The possibility of directly viewing acute myocardial ischemia is patently a very challenging prospect and for this reason is being pursued actively.

For other parts of the body, many possibilities readily come to mind: the detection of infarctions in the abdominal organs, cirrhosis and fatty degeneration of the liver, ascites, transudates and exudates, and neoplasms of the gut and generative organs are just a few. Not least among these is the direct detection of that most elusive of tumors, carcinoma of the pancreas; density measurements indicate that this also should eventually be realizable.

Moreover, there are a number of different ways of looking through this "window" which could enhance its potential as a diagnostic and research tool. One of these is selective enhancement of the contrast of a tissue by injection of stable isotopes, e.g., heavy water, or inhalation of ^{18}O, ^{13}N, or other physiologically compatible substance. These would be metabolized like the normally abundant isotope and would not pose a radiation problem. Here also there are intriguing possibilities, such as using two beams of different energies to probe in quick succession along a given path-length, or of two narrow beams to probe along closely adjacent paths. Without too much of a stretch of the imagination, though admittedly reserved for the distant future, one can consider that, in theory, these methods could supply first-order information on the chemical composition of the body or parts of the body, the pH of tissues, and the "micro-structure" of closely placed body organs or parts of organs.

Clearly it would be exciting to perform such analyses in the intact living organism for the purposes of diagnosis and research, but the drawback is that their realization poses the problem of very great technological sophistication. In addition, because the body maintains a rather constant *milieu interieur*, only extremely fine differentials, very difficult to detect, would be of interest.

How useful proton and heavy-ion radiography will be in diagnosis will depend very much on the way in which technique fits into the ever-increasing array of diagnostic tools available to the clinician. This not only applies to a comparison with x-rays and the techniques of nuclear medicine, but to the numerous immunologic, chemical, cytologic, and endoscopic tests that are being developed. However, as a non-invasive method with high "visibility" and a wide margin of safety, it promises considerable potential for early diagnosis. In this sense, it may be said to approach an "ideal" diagnostic tool.

THE FUTURE DEVELOPMENTS OF HEAVY ION RADIOGRAPHY

The final question which may be asked is "How, by what means, or in what direc-

tion is proton and heavy-ion radiography likely to develop?" Obviously much of this will depend upon suitable technology and on clinical need.

With regard to a suitable technology, the prime aim would be "to bring the particles to the patient" in a hospital setting; this is eminently possible. According to Dr. R. L. Martin, Director of the Accelerator Research Division, Argonne National Laboratory, it would be well within the state of the art to build a simple proton synchrotron made up of a ring of magnets arranged in a 21-foot diameter circle. This synchrotron would accelerate negative hydrogen ions which, by simply striking a thin foil at the appropriate moment, would be broken up into their constituent electrons and protons. The latter would then emerge as separate beams by which up to six patients could be scanned simultaneously. This machine would require little, if any shielding, and would operate on batteries which could be recharged overnight. As a production model, moreover, it should cost no more than $300,000. This alone makes it very competitive with some of the highly specialized x-ray equipment used today.

Accelerators to produce the heavier ions, as indicated earlier, would be much more expensive; but if experience and need dictate, these also can be built.

At the detector level, there is likewise nothing beyond the state of the art, and film or instrumented imaging techniques would be employed according to their appropriateness. The latter would employ methods well established in the field of particle physics; these methods would overcome the limitations inherent in film, prevent the loss of information, and yet permit image-processing. Moreover, quantum detection efficiency could be readily achieved, with the important consequence of minimizing the dose to the patient.

Yet another possibility, and one which addresses itself to a very practical problem, is "how to get the patient out of the water box," i.e., compensating for variations in body shape without immersion in fluids. This could be accomplished by utilization of the methods of non-coherent optical techniques for surveying surfaces.

Such methods have the triple advantage of adequate precision, capability of digital processing, and of being feasible with equipment which does not have to be interposed in the path of the heavy ions.[15] However, the recent emergence of the EMI whole body scanner indicates, that for x-rays at least, computer programs are directly able to take care of variations in body contour. Possibly these could be adapted for proton radiography, though detector design would still have to cope with large variations in particle range.

Therefore, at the purely technological level there appears to be no major stumbling block, and from this point of view the widespread use of the method would appear assured. Nonetheless, the building of machines, albeit important, is but a first necessary step for the accumulation of data. The larger and more protracted problem is the interpretation of these data. Here we may anticipate that a situation analogous to that in x-radiography will prevail, where correlations between the radiologic, clinical, and pathologic aspects of a case will enable the proper evaluation of a density anomaly. Knowledge thus gained would aid development of a refined instrumentation; but, also important, the ideas of heavy-ion radiography would be made more consonant with the canons of our times. The history of science teaches that this is necessary if any new concept is to gain full acceptance.

How does one truly test the validity and worth of a new method such as this? It goes without saying that the objective throughout is to produce images of the highest diagnostic quality. As has been pointed out, this goal may not necessarily coincide with the production of images that are the most faithful from the physical standpoint.[16] Many intangibles are involved, and thus the physical methods, e.g., modulation transfer functions, etc., currently being developed for the objective transfer of diagnostically important in formation with x-rays have a role to play here. Yet the matter goes beyond

purely physical considerations; one is rapidly faced with the psychophysics of perception. This aspect has not escaped the attention of the x-radiologists, and the techniques which they have applied in recent years, such as the receiver-operator curve (ROC), should be useful.[17] Such methods appear very powerful, and by their application heavy-ion radiography should be able to escape the fate of an empirical evaluation.

Generally, therefore, a diversity of developments is required, but with their aid a new "window" will be realized for investigation of the living body. We have reason to believe that this new "window" of proton and heavy-ion radiography will prove as valuable as x-radiography is at present. It should provide another powerful tool for the radiologist in his fight against disease.

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SYSTOLIC TIME INTERVALS IN A HEALTHY ADULT POPULATION: USE OF TWO-CHANNEL RECORDER FOR SURVEYS

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ABSTRACT. Left ventricular function was determined in 321 healthy insurance company employees by use of indirect systolic time intervals. Comparison of subgroups was made according to sex and three age ranges: 16 to 34 years, 35 to 49 years, and 50 to 64 years. Systolic time intervals included left ventricular ejection time (LVET), pre-ejection period (PEP), total electromechanical systole ($Q\text{-}S_2$) and PEP/LVET ratio.

Mean values for each systolic time interval increased from the youngest to the oldest age subgroup in both sexes. These findings were associated with a decreased mean heart rate with increasing age. All systolic time intervals were consistently longer at similar heart rates in the oldest vs. youngest group of women. Similar age comparison of systolic time intervals for men showed longer PEP for the oldest group at all heart rate ranges studied, and a longer $Q\text{-}S_2$ and greater PEP/LVET ratio for the oldest group at most heart rate ranges.

This technique may be useful for evaluation of left ventricular performance in surveys of large population groups.

INTRODUCTION

In the past decade there has been a growing interest in external recording techniques to evaluate left ventricular function. Systolic time intervals derived from simultaneous recordings of the electrocardiogram, phonocardiogram and external carotid pulse have correlated with left ventricular performance data obtained by cardiac catherization.[1-4] Indirect measurements of cardiac performance can be obtained rapidly and easily and are therefore useful for serial studies of large patient groups.

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Data from studies using systolic time intervals have demonstrated that abnormalities in these intervals occur with impaired left ventricular performance.[5] Changes in preload (left ventricular filling) and afterload (resistance to left ventricular outflow) may also affect systolic time intervals.[4,6] In normal hearts, there may be changes in externally recorded systolic time intervals due to variations of heart rate, patient position, hematocrit, medications, and sex and age differences.[7,8]

Most systolic time interval determinations have been performed using high-speed recordings (100 mm/sec) which allow reliable measurements to the nearest 5 msec. However, determination of left ventricular ejection time from the indirect carotid pulse tracing at ordinary electrocardiographic paper speed (25 mm/sec) produces regression equations similar to those from higher speed recordings.

This investigation was designed to determine (1) whether two-channel recording devices commonly used outside the

hospital and research laboratory could usefully monitor left ventricular performance in large groups of patients by measuring systolic time intervals; (2) whether regression line equations for systolic time intervals derived from this technique correlate with results produced by more sophisticated techniques; and (3) whether this technique could determine sex-and-age-related variations in a healthy population. To make these determinations we studied systolic time intervals in 321 healthy men and women, insurance company employees, aged 16 to 65.

MATERIAL AND METHODS

Initially, 350 persons employed at the Equitable Life Assurance Society volunteered for these studies. Of these, 29 were excluded because of (1) systemic hypertension (diastolic pressure—100 mm Hg); (2) abnormal heart sounds or murmurs; (3) evidence of enlarged heart on chest x-rays; (4) abnormal electrocardiogram; (5) systemic disease in other organs which might affect cardiac performance; and (6) use of medications which might affect cardiac performance. The remaining 321 were considered to have no evidence of cardiovascular disease and were included in the final study. The following grouping was used for evaluation of the population studied:

Group I: 160 Men
- Subgroup 1A: 57 Subjects (ages 16 to 34)
- Subgroup 1B: 51 Subjects (ages 35 to 48)
- Subgroup 1C: 51 Subjects (ages 50-65)

Group II: 161 Women
- Subgroup 2A: 60 Subjects (ages 16-34)
- Subgroup 2B: 50 Subjects (ages 35-49)
- Subgroup 2C: 51 Subjects (ages 50-65)

Subjects were evaluated after 10 minutes rest in the supine position. Systolic time intervals were recorded at 50 mm/sec paper speed using a twin-channel heat stylus Sanborn recorder. Recording was first made of 10 consecutive simultaneous electrocardiographic and phonocardiographic complexes. Immediately following this, 10 consecutive electrocardiographic and external carotid pulse complexes were recorded.

The external carotid pulse was recorded using a 2cm diameter funnel held by hand, coupled with a 15cm long, 2mm diameter rubber tube to a piezo-electric crystal. There were virtually no differences in heart rate between recordings of the two sets of complexes for each patient.

Systolic time intervals were measured in the usual manner.[1] Mean value of the 10 consecutive complexes was used for determining each time interval. The following intervals were measured: (1) total electromechanical systole ($Q\text{-}S_2$), measured from the beginning of the QRS complex to the aortic component of the second heart sound identified as the 1st high frequency deflection of the phonocardiogram; (2) left ventricular ejection time (LVET), measured from the upstroke to the incisura of the indirect carotid pulse; (3) pre-ejection period (PEP), derived by the formula: $PEP = (Q\text{-}S_2) - (LVET)$. In addition, PEP/LVET ratio for each subject was derived from the mean values for these two intervals (Fig. 1).

Regression lines for $Q\text{-}S_2$, LVET, and PEP plotted against heart rate were determined for each subgroup. In addition, mean values for systolic time intervals were determined and compared according to age differences within each sex group and sex differences at each age range. Finally, mean systolic time intervals were compared at similar heart rates to determine age and sex differences. Statistical significance of mean values was determined using student's two-tailed t-test, using 95 percent confidence limit ($p < .05$).[10] Calculations were made on Olivetti Programma 101 and Wang 700 computers.

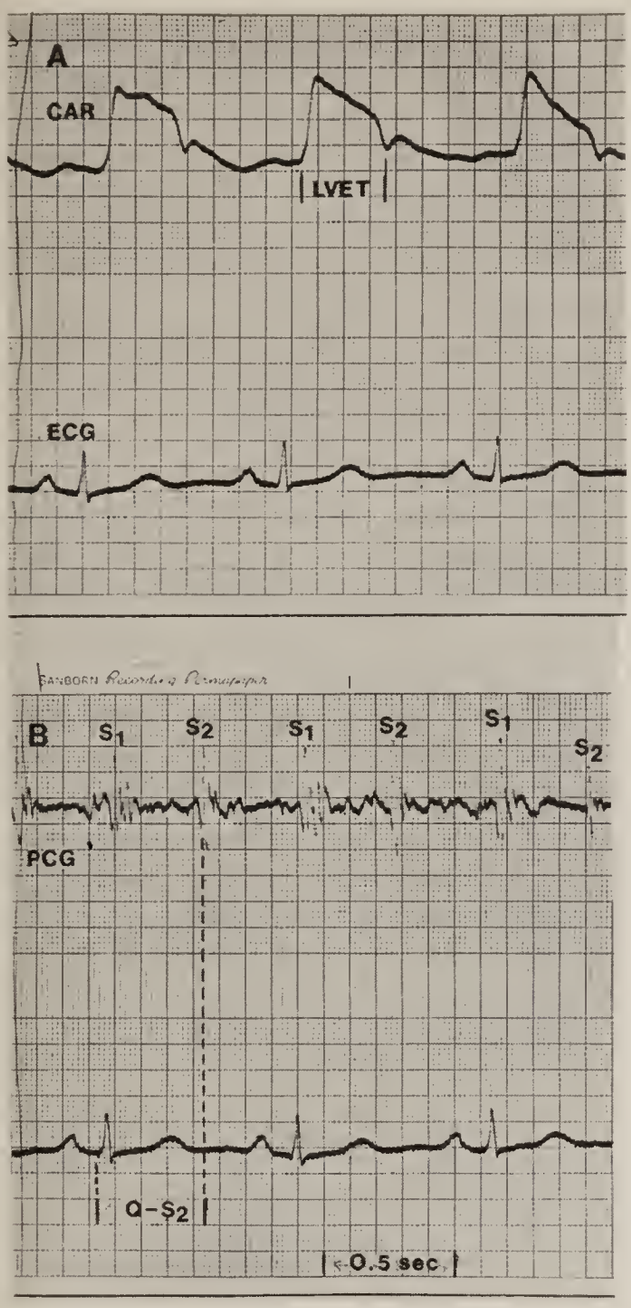

Fig. 1—Recording speed is 50 mm/sec. In the upper panel the ECG (Lead II) and carotid pulse tracing (CAR) are recorded simultaneously. In the lower panel the ECG and phonocardiogram (PCG) tracing are recorded simultaneously. LVET is determined from the tracing at the top and Q-S_2 from the tracing at the bottom. PEP is determined by subtracting LVET from Q-S_2.

RESULTS

Mean Values For Systolic Time Intervals by Age Group Comparison

Heart rate and systolic time intervals for each age subgroup are shown in Table 1. Older women (2C) had a slower heart rate than younger ones (2A). There were no differences in the mean heart rates of men. Systolic and diastolic pressures increased with age, and also were higher than in women of each age group.

Mean arterial pressure was greater for older subjects at all heart rate ranges for both sexes. These differences were noted in three of the four heart rate groups for men and two of the three heart rate groups for women. There was no correlation between any systolic time interval and mean arterial pressure.

Mean LVET for men was no different in intergroup comparisons. The older group of women had longer LVET than the younger group (2A v. 2C) $p. < .001$. Mean PEP also increased progressively with age in both sexes. For both men and women, PEP was higher in the older group than in the younger group. Mean Q-S_2, reflecting the sum of PEP and LVET, also increased progressively with age in both sexes, and the mean values were higher in the older group of both sexes. There were no significant differences in PEP/LVET ratios with age.

Comparisons between men and women at the same age range showed a greater heart rate for younger women (2A), and a greater LVET for women in the middle age group, when compared with men (1B v. 2B). Q-S_2 was larger in the middle and older age groups of women than in men.

Regression Lines For Ejection Time Vs. Heart Rate

Regression lines were calculated for individual systolic time intervals vs. heart rate (Table 2). An inverse correlation for LVET and Q-S_2 with heart rate was noted for each age group of both men and women. Although inverse correlations were also noted for PEP vs. heart rate for the entire group of men and of women, only the younger group of men (1A) displayed an inverse correlation when individual age subgroups were evaluated.

Except for PEP, the slopes of the regression equations increased with older age for both men and women, and the slope was steeper in each age group for men, as compared with that of women.

Faster heart rate produced shorter LVETs for men and women; but men showed greater abbreviation of this inter-

val at higher rates (Fig. 2). Women had a longer LVET at faster heart rates than men, in both younger and older age groups.

The slopes for PEP were similar in the entire group of men vs. women (Fig. 3). For any heart rate, the PEP estimated by the regression equations was longer for women than for men.

For men and women, Q-S_2 lengthened progressively with age (Fig. 4). Analysis of regression lines for the youngest and oldest age groups of women demonstrated differences in Q-S_2.

Comparisons Based on Similar Heart Rate

Results of systolic time interval comparisons between the youngest and oldest male and female groups at similar heart rates are noted in Table 3.

For men, systolic time intervals were no different in the two extreme age groups at heart rates of 50-59, 60-69, 70-79, and 80-89 beats per minute. Mean PEP was longer in the older female group at 70 to 79 beats per minute. Q-S_2 was also longer for the older female group at two of the three heart rate ranges. PEP/LPET ratio was increased in older women at 70 to 79 beats per minute.

Too few subjects had heart rates above 90 to allow statistical evaluation above this rate.

DISCUSSION

Systolic time intervals reflect changes in left ventricular function, but few studies have been made to determine changes in these intervals with age, in a normal non-hospital population.

The Q-S_2 and LVET regression line equation in this study show an increase in slope, with increasing age, for both sexes. This may be due partially to the decrease in mean heart rate with age, which has been shown to increase the slope of the regression line for LVET.[11] Other factors which may account for longer LVET in older persons include (1) increased pe-

TABLE I

SYSTOLIC TIME INTERVALS: Grouped According to Age and Sex
(Mean ±1 Standard Deviation)

MEN	(mm Hg) SYST. PR.	(mm Hg) DIAST. PR.	HR	msec LVET	msec PEP	msec Q-S_2	PEP/LVET
1A (n=57)	120 ± 12	73 ± 9	74 ± 14	279 ± 22	86 ± 16	364 ± 29	.309 ± .06
1B (n=51)	122 ± 13	77 ± 9	72 ± 11	280 ± 22	91 ± 17	371 ± 26	.325 ± .07
1C (n=52)	134 ± 18	80 ± 9	69 ± 11	285 ± 30	93 ± 21	379 ± 33	.326 ± .08
WOMEN							
2A (n=60)	111 ± 10	69 ± 13	80 ± 13	279 ± 20	88 ± 18	367 ± 23	.315 ± .08
2B (n=50)	115 ± 14	73 ± 10	76 ± 12	290 ± 23	94 ± 17	384 ± 26	.324 ± .07
2C (n=51)	125 ± 16	76 ± 1	72 ± 10	295 ± 22	99 ± 17	394 ± 24	.336 ± .08
COMPARISON OF MEAN VALUES Student's 2 tailed-T-Test							
1A v. B	NS	<.02	NS	NS	NS	NS	NS
B v. C	<.001	>.05	NS	NS	NS	NS	NS
A v. C	<.001	<.001	>.05	NS	<.05	<.02	NS
2A v. B	NS	>.05	>.05	<.02	>.05	<.001	NS
B v. C	<.001	NS	NS	NS	NS	NS	NS
A v. C	<.001	<.005	<.001	<.001	<.001	<.001	>.05
1 v. 2A	<.001	>.05	<.01	NS	NS	NS	NS
B	<.02	<.05	>.05	<.05	NS	<.02	NS
C	<.01	<.05	NS	NS	NS	<.05	NS

Abbreviations: LVET = left ventricular ejection time
PEP = pre-ejection period
NS = not significant

ripheral resistance (2) loss of aortic wall elasticity and (3) changes in rate of myocardial fiber shortening.

Although there was some variation in the regression lines, the differences were small. Willems, et al.[12] have indicated that in a much older population (60 to 69 years) there is an increase in LVET with aging, independent of changes in heart rate and blood pressure, with a longer interval in women compared with men. In our study, an increase in mean values for LVET in older women when compared with the younger group probably reflected the lower mean heart rate in the older group. However, the LVET in middle-aged women was increased over that of middle-aged men, despite a higher rate in this group of women. This speaks for an effect independent of heart rate. In all three of our age groups, mean heart rate was greater in women, but LVET was equal to or greater than that of men.

We could find no correlation of systemic arterial pressure with systolic time intervals, although others have found a small effect on LVET and PEP in conditions of changing afterload.[13,14] Certainly the mean arterial pressure increased with age in both sexes and this might have affected PEP and LVET at higher heart rates in the older population. In our study PEP/LVET ratios increased in both men and women with increasing age, although this did not achieve statistical significance.

Linear regression data for LVET has been demonstrated by Spodick et al. at recording speeds of 25 mm/sec, one-half the speed of our tracings.[9] Since most electrocardiographic machines can record at a speed of either 25 or 50 mm/sec, we used the latter speed to demonstrate that regression line values could be obtained and result in equations similar to those obtained by higher speed recorders (Table 4).

TABLE II

LINEAR REGRESSION EQUATIONS: Systolic Time Interval v. Heart Rate

Interval	Age Group	No.	Equations	R	P
LVET	All Men	160	ET = −1.58 HR + 393	−0.77	<.001
	1A (under 35)	57	ET = −1.22 HR + 370	−0.77	<.001
	1B (35-49)	51	ET = −1.48 HR + 386	−0.74	<.001
	1C (50-65)	52	ET = −2.19 HR + 436	−0.81	<.001
	All Women	161	ET = −1.36 HR + 391	−0.75	<.001
	2A (under 35)	60	ET = −1.12 HR + 368	−0.73	<.001
	2B (35-49)	50	ET = −1.47 HR + 401	−0.77	<.001
	2C (50-65)	51	ET = −1.54 HR + 406	−0.70	<.001
PEP	All Men	160	PEP = −0.35 HR + 115	−0.24	<.002
	1A (under 35)	57	PEP = −0.57 HR + 127	−0.49	<.001
	1B (35-49)	51	PEP = −0.36 HR + 116	−0.23	NS
	1C (50-65)	52	PEP = −0.05 HR + 97	−0.03	NS
	All Women	161	PEP = −0.29 HR + 115	−0.20	<.01
	2A (under 35)	60	PEP = −0.28 HR + 111	−0.21	NS
	2B (35-49)	50	PEP = −0.08 HR + 100	−0.05	NS
	2C (50-65)	51	PEP = −0.18 HR + 112	−0.10	NS
Q-S_2	All Men	160	Q-S_2 = −1.92 HR + 509	−0.78	<.001
	1A (under 35)	57	Q-S_2 = −1.71 HR + 490	−0.81	<.001
	1B (35-49)	51	Q-S_2 = −1.83 HR + 502	−0.76	<.001
	1C (50-65)	52	Q-S_2 = −2.24 HR + 534	−0.75	<.001
	All Women	161	Q-S_2 = −1.67 HR + 508	−0.76	<.001
	2A (under 35)	60	Q-S_2 = −1.39 HR + 479	−0.78	<.001
	2B (35-49)	50	Q-S_2 = −1.55 HR + 502	−0.72	<.001
	2C (50-65)	51	Q-S_2 = −1.71 HR + 518	−0.71	<.001

Abbreviations: LVET = left ventricular ejection time
PEP = pre-ejection period
Q-S_2 = total electro-mechanical systole
R = coefficient of correlation
P = significance of correlation

TABLE III

COMPARISONS OF SYSTOLIC TIME INTERVALS BY MATCHED HEART RATES
Younger and Older Groups (15-34 yrs. v. 50-65 yrs.)
(Mean ±1 Standard Error of Estimate)

MALES	Group	No.	Heart Rate	MABP	LVET	PEP	Q-S_2	PEP/LVET
HR: 50-59	1A (15-34)	(10)	55 ± 1	88 ± 3	302 ± 4	92 ± 6	394 ± 7	.304 ± .018
	1C (50-65)	(9)	56 ± 1	101 ± 5	313 ± 4	94 ± 10	408 ± 10	.302 ± .029
	Significance:		NS	P<.05	.1>P>.05	NS	NS	NS
60-69	1A	(10)	65 ± 1	87 ± 2	296 ± 3	92 ± 4	388 ± 4	.311 ± .013
	1C	(11)	64 ± 1	99 ± 4	295 ± 2	96 ± 6	392 ± 7	.326 ± .021
	Significance:		NS	<.05	NS	NS	NS	NS
70-79	1A	(15)	74 ± 1	85 ± 2	278 ± 4	80 ± 4	358 ± 4	.290 ± .016
	1C	(18)	73 ± 1	95 ± 2	280 ± 5	89 ± 4	369 ± 5	.304 ± .017
	Significance:		NS	.005	NS	NS	NS	NS
80-89	1A	(13)	84 ± 1	93 ± 3	260 ± 4	83 ± 5	343 ± 5	.322 ± .020
	1C	(8)	84 ± 1	98 ± 4	248 ± 11	93 ± 9	340 ± 8	.387 ± .050
	Significance:		NS	NS	NS	NS	NS	NS
FEMALES								
HR: 60-69	1A	(12)	66 ± 1	85 ± 2	291 ± 4	98 ± 6	388 ± 6	.337 ± .022
	1C	(18)	63 ± 1	91 ± 3	308 ± 5	101 ± 5	409 ± 2	.349 ± .027
	Significance:		NS	NS	.1>P>.05	NS	<.005	NS
70-79	1A	(18)	76 ± 1	82 ± 2	286 ± 4	84 ± 5	370 ± 2	.300 ± .021
	1C	(17)	74 ± 1	93 ± 3	291 ± 3	104 ± 3	395 ± 3	.358 ± .012
	Significance:		NS	<.005	NS	<.005	<.001	<.025
80-89	1A	(16)	84 ± 1	85 ± 2	273 ± 3	90 ± 4	363 ± 3	.325 ± .017
	1C	(11)	84 ± 1	92 ± 2	277 ± 5	94 ± 4	371 ± 7	.339 ± .018
	Significance:		NS	<.025	NS	NS	NS	NS

Student's -2 tailed -t test used.
Abbreviations: MABP = mean arterial pressure (⅓ systolic-diastolic + diastolic)
No. = number of subjects in group
LVET = left ventricular ejection time
PEP = pre-ejection period
NS = not significant

TABLE IV

SYSTOLIC TIME INTERVAL REGRESSION LINES V. HEART RATE IN OTHER STUDIES
(Paper Recording Speed 100 mm/sec)

LVET	1. Weissler (1969)[2]	M LVET = −1.7 HR + 413
		F LVET = −1.6 HR + 418
	2. Diamant (1970)[3]	LVET = −1.3 HR + 380
	3. Willems (1970)[12]	M LVET = −1.82 HR + 428*
		F LVET = −1.66 HR + 424*
PEP	1. Weissler (1969)	M PEP = −0.4 HR + 131
		F PEP = −0.4 HR + 133
	2. Diamant (1970)	PEP = −0.3 HR + 110
	3. Hamosh (1972)[15]	PEP = −0.4 HR + 126
$Q\text{-}S_2$	1. Weissler (1969)	M $Q\text{-}S_2$ = −2.1 HR + 546
		F $Q\text{-}S_2$ = −2.0 HR + 549
	2. Diamant (1970)	$Q\text{-}S_2$ = −1.7 HR + 490
	3. Hamosh (1972)	$Q\text{-}S_2$ = −2.0 HR + 522

*Older "normals": (Age range 60-90). M = males. F = females.
No sex differentiation when regression line was calculated from both sexes.
Superior numbers refer to references.

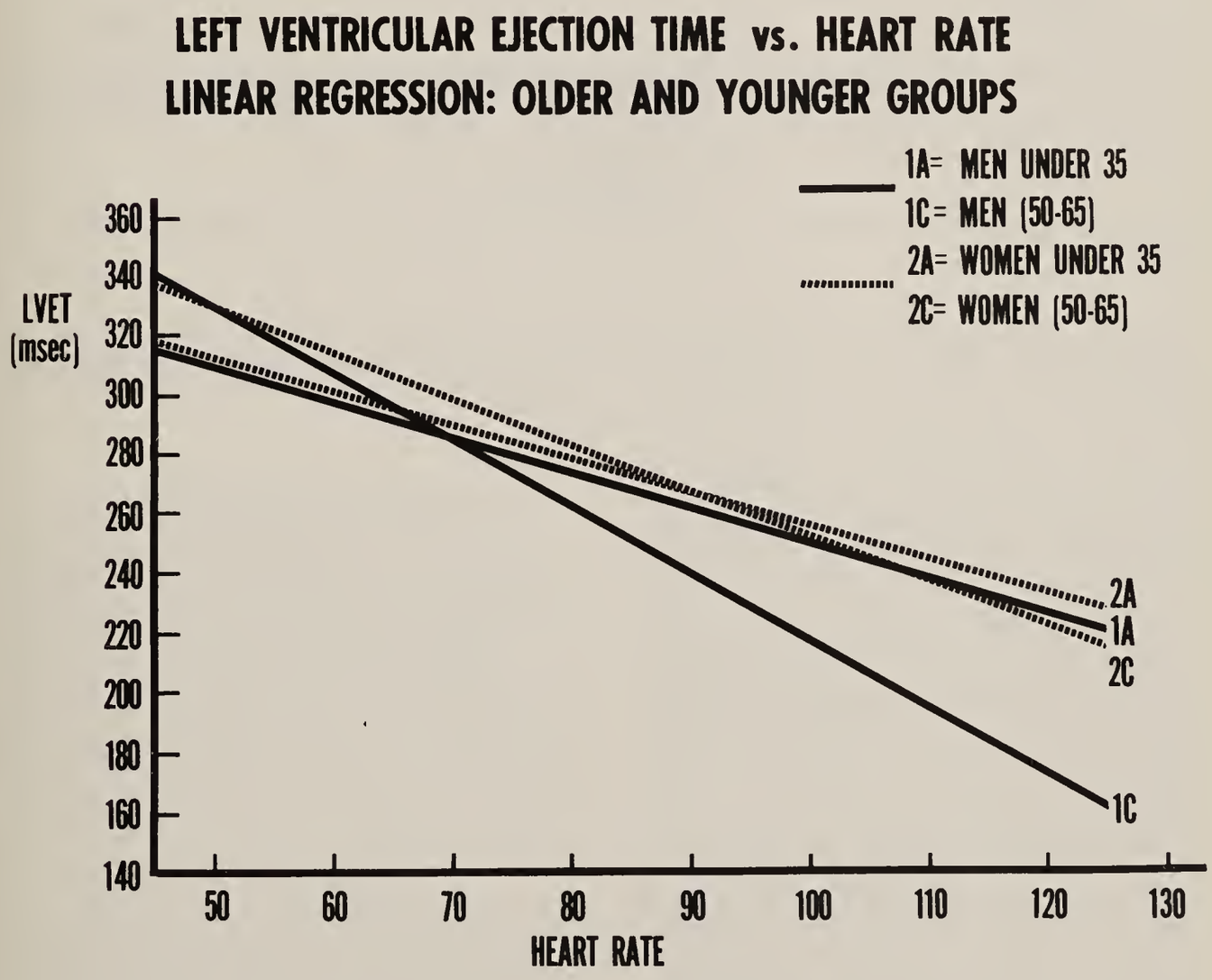

Fig. 2—At faster heart rates, LVET is shorter for the older groups than the younger groups, and women have a longer LVET than men.

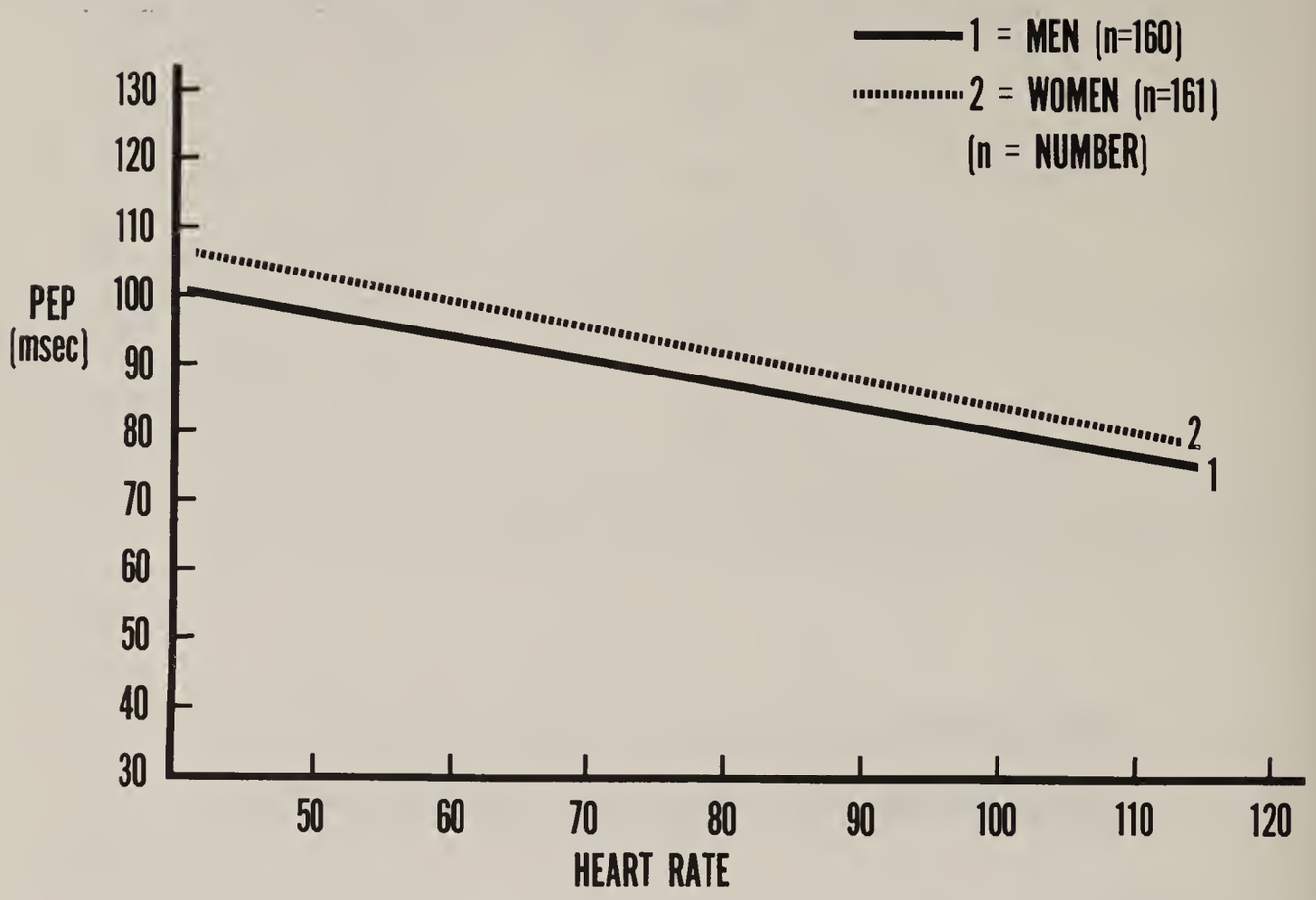

Fig. 3—The slope is similar, but women have a longer PEP throughout the range of heart rates than men.

The most accurate data can be obtained from Q-S_2, since this is the longest interval. LVET measurement, dependent on carotid upstroke and incisura, can also be determined at lower speeds, as previously demonstrated by Spodick et al.[9] PEP, a derived value, and approximately one-third the duration of LVET, may be more susceptible to error at this low speed. Our data show an inverse correlation between PEP and heart rate in younger men, and in both sexes only when the entire age range is used for the correlation.

This study and other studies using STI suggest that the techniques might be of use in large scale evaluation of left ventricular function in routine screening of employees. Individual STI determinants could be compared with normal regression lines of STI values at each heart rate in order to determine abnormalities of function. Serial studies might allow early diagnosis of dysfunction of the left ventricle.

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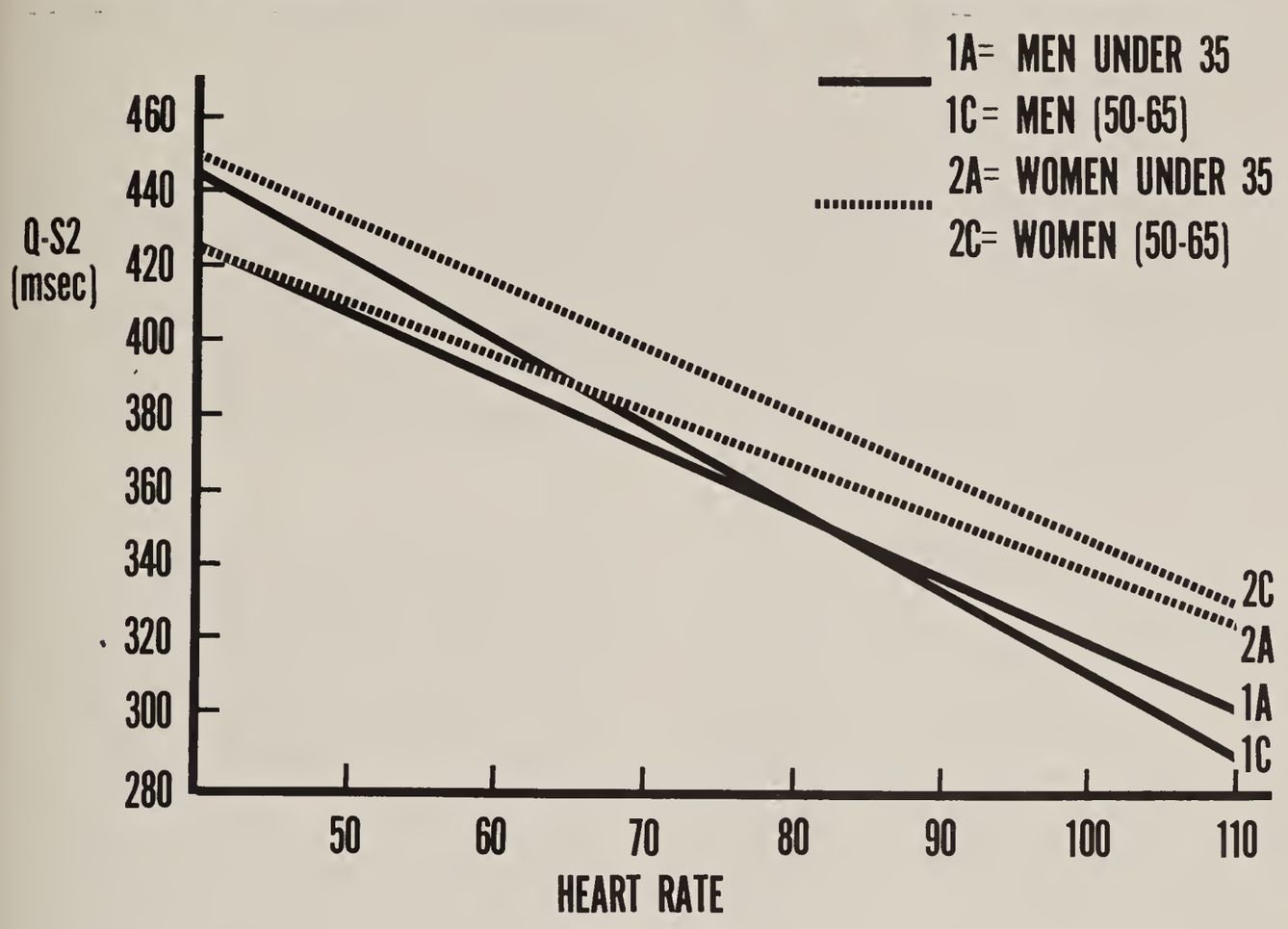

Fig. 4—At faster heart rates, the Q-S_2 for older men is shorter than the expected value for younger men, while it is longer for older women than younger women. Also, at the upper range of heart rates, Q-S_2 for women is longer than the values for men.

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THE CLINICAL ASSESSMENT OF ACUTE SUICIDAL POTENTIAL: A REVIEW

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ABSTRACT. This review suggests that effective prevention of suicide depends to a large extent on the recognition of both chronic and acute signs of high suicidal potential. Observations from the available clinical literature are reviewed in a consideration of both traditional as well as less commonly taught clinical signs of an impending suicide. A critical discussion of the relative value of these signs and symptoms emphasizes the important distinction between chronic and acute factors of suicidal potential as well as the importance of emphasizing clusters of pre-suicidal signs.

INTRODUCTION

The current state of suicide assessment deserves more attention. There has been much theorizing but few documented clinical observations. In the literature, there is little information available to the clinician for the assessment of immediate suicide risk. Methodological deficiencies of available studies have led to overgeneralizations from suicidal behavior of low lethal intent to that of high intent.[1,2] This paper critically reviews the literature on suicide prediction as well as newer clinical approaches, in order to find useful indicators which might help in the preventive prediction of suicide at this stage of our knowledge.

Unfortunately, the physician is sometimes in the position of being the only person with the opportunity of recognizing suicidal intent. Studies have shown that from 40 to 75 percent of suicidal individuals will see a physician within six months to a year preceding their self-destructive acts.[3-9] A number of studies have pointed out that even while receiving psychiatric treatment, psychiatric hospitalization,[10-16] or treatment with psychotrophic drugs,[17,18] patients do commit suicide. Consequently it is of utmost importance that the physician, and especially the psychiatric clinician, develop skills in recognizing the potentially serious suicidal patient.

It has been suggested that suicide can be a logical, rational decision based on an individual's reality-situation.[19-22] The evidence, however, seems to support the contention that most suicides occur in the context of psychiatric illness.[23-35] Brown,[2] has concluded that there is a greater reduced incidence of psychiatric illness associated with suicide when the data is more closely examined. This apparent discrepancy perhaps can be explained by the fact that the criterion for mental disturbance in the five studies reviewed by Brown was whether the individual had been or was currently undergoing psychiatric treatment. His studies also reported that a high percentage of individuals judged not to be psychiatrically ill, had

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seen or were currently involved with a medical doctor at the time of their suicide.[36,37] Occurrences of suicide have been, therefore, reported in "masked depression"—the depressed mood is less apparent, being reflected by chronic or acute somatic complaints or psychosomatic disorders.[6,8,38-40] The absence of psychiatric treatment at the time of suicide, does not necessarily preclude the existence of a serious mental disturbance. Furthermore, it has been observed that severely depressed patients may appear symptom-free just prior to suicide.[41,42] This may lead to an erroneous assumption that the individual is "normal" at the time of suicide. While suicidal behavior may manifest itself in patients fitting any psychiatric diagnostic category, it has been found most prevalent among middle-aged patients in depression,[42-47] especially manic-depression and psychotic depression,[27] as well as in alcoholism,[48-53] and in schizophrenia, especially in younger age groups.[38,54-58] Typically, the middle-aged patient with symptoms of a serious depressive syndrome manifesting such signs as sleep disturbance, weight loss, dry mouth, loss of sexual drive, gastrointestinal discomfort, complete loss of interest, impairment of function, delusional guilt, neglect of personal appearance and cleanliness, inability to make decisions, a feeling of emptiness, psychomotor retardation or agitation[59] in a depressed mood, characterized by feelings of hopelessness and helplessness, is a high suicide risk. In general, the risk of suicide appears to be greatest in the early course of depressive illness, and decreases as drive and affect is "burned out" and where life becomes a kind of partial death, without ambition and seemingly without purpose.[60]

PRE-LETHAL FEATURES

The following sections are predicated on the view that the effective recognition of the pre-suicidal patient will depend on a synthesis of acute pre-lethal factors occurring in the context of chronic pre-lethal features in the presence of one or more situational precursors of suicide which commonly precede self-destruction. We describe those characteristics emerging from the literature sources independently, which represent suicidal individuals. It is acknowledged, however, that these features probably will not apply to every suicidal patient. Additional research will delineate features that seem to identify the high risk individual. Clinical assessment must rest on the presence of a number of pre-lethal variables both acute and chronic, and not on one or two isolated observations.

Table I lists some of the features that may characterize the chronically suicidal individual:

1) Repeated communication of a wish to die or suicidal thoughts can be regarded as one feature of the chronically suicidal person.[27,38,61-68] However, this in itself is not sufficient to distinguish the high- from the low-risk patient, since it has also been observed that the majority of the much larger group of patients who attempt but do not complete suicide also convey intent in advance.

2) The recent literature recognizes intense dependency as an underlying dynamic in the suicidal individual.[69-77] This dependency has been observed to be pervasive throughout all spheres of the suicidal individual's life style where inordinately excessive demands are made on others for constant attention, affection and approval, and where the individual feels unable to cope for himself, thereby needing continual supervision and guidance.[78] Others have independently observed this basic feeling of helplessness in patients who commit suicide.[79-81]

3) Tendencies toward rigid thinking which does not allow for alternatives in a crisis, and bipolar dichotomous thinking, (to think in opposites) have been observed in the personalities of many suicidal patients.[61,82-92]

4) A related but distinct personality feature, to rigidity, is pathological perfectionism.[32,55,70,90,92-95] This trait finds expression in an anxious striving towards perfection in all undertakings.

5) Another personality feature closely related to both rigidity of thought and pathological perfectionism is the obsessive-compulsive mode of adjustment.[3,8,32,61,91,96-99] However, some authors while characterizing the pre-morbid personalities of the typical depressed patient as having obsessive features, claim that obsessive-compulsive behavior itself can be a defense against suicide.[30,39,89,100] This apparent contradiction might be explained by the observation that at the point when the obsessive-compulsive defense fails, perhaps leaving a rigid personality undefended, the suicidal risk increases.[100]

6) A less commonly recognized characteristic repeatedly associated with a high risk of suicide is paranoia,[61,67,98,101-112] While paranoia can serve as a temporary defense against depression, unrecognized suicidal impulses may result when this defense fails.[18,61,96,101] A minority of studies reported no evidence of paranoia in suicidal subjects.[31,113,114]

7) As opposed to Freud's views of suicide and depression as unconscious rage toward a lost loved object turned back on the self,[115] recent reviews of clinical cases have suggested a rather high frequency of externalized anger and even violent tantrums in the histories of patients who commit suicide or make serious attempts.[93,116-125]

8) A review, therefore, of the personality features of the seriously suicidal patient commonly reveals evidence of intermittent periods of loss of control over psychic functions, especially related to outbursts of rage or tantrum-like behavior.[38,59,62,99,116,126-128] Alcohol and other drugs may lower the threshold in these patients, facilitating loss of control.[3,39,85,129] Whereas this characteristic is not uncommon in paranoid individuals, the cluster of loss of control in combination with the presence of obsessive-compulsive and rigid personality traits is less common and therefore this syndrome may be particularly useful. Emphasis is placed on the recognition of clusters of these variables rather than on the presence of any one feature in the assessment of suicide risk. With respect to loss of control, it should be noted that Freud[115] observed mental regression as a frequent feature among patients committing suicide.[130,131] Spiegel and Neuringer[131] state that a regression in mental function may be necessary to overcome the dread of death in order to make a serious suicide attempt.

9) An infrequently noted but perhaps important feature is the high chaotic energy level and stimulus-seeking aspect of some suicidal individuals.[132,133] It has been observed that actual suicidal individuals may be described as possessing high energy which however is expressed in random or explosive outbursts toward unrealistic goals.[132]

10) Perhaps the most important characteristic of the chronically high-risk patient is that of impaired capacity for interpersonal relating.[30,53,61,134-141] This feature of interpersonal distance is one of the most useful clinical signs of the high-risk suicidal patient in our experience, both in terms of prediction and prevention. Rubenstein, et al.[142] noted that suicide attempts of high lethality tended to be those where no interpersonal aim could be found, as compared to the low lethality attempts in which interpersonal gains were clearly the object of the behavior. In addition, follow-up studies have described two separate but overlapping populations of patients, one large group who make repeated gestures, and a smaller group who use lethal methods and usually kill themselves on the first or second try.[143,144] Many in this lethal group have either a protracted course or one of the recurrent depressive episodes always associated with suicidal preoccupation.

A recent study has shown that 91 percent of the completed suicides made no attempt to communicate their intent just prior to their suicide, while the suicide-gesture group contacted a significant other 73 percent of the time.[145] Thus, it is not uncommon for suicide to occur in the presence of others. The dramatic gestures of a psychotic individual may herald a serious suicide risk which is confirmed in subsequent behavior.[3,145]

TABLE I

CHRONIC PRE-LETHAL FEATURES

1. Suicidal communications
2. Symbiotic dependency with reliance on external controls
3. Rigid thinking
4. Paranoid traits
5. Externalized anger
6. Intermittent loss of control
7. Chaotic stimulus seeking
8. Impaired interpersonal coping

A study of 30 patients of varying degrees of suicidal risk[135] indicated a high incidence of certain interpersonal characteristics in patients who made high lethality attempts, which have independently been recognized by other investigators: a) Interpersonal incapacity or the inability to maintain warm mutual interdependent relationships, with the resulting high-risk sign of isolation.[30,120,132,139,146,147] b) Marital isolation: Long-standing interpersonal isolation and disengagement in spite of overt appearances of conventional marriage, i.e., "emotional divorce."[30,51,78,85,86,96,148,149] c) Distorted communication of dependency wishes: The inability of the individual to express directly his dependency to significant others in a manner which might lead to support and gratification.[27,73,75,150-152] and d) Help negation: Defined as a specific form of help rejection wherein the patient persistently withdraws from, terminates, or denies any help or relationships with staff or significant others.[5,30,46,93,127,153,154]

Although patients who are depressed tend to withdraw from interpersonal relationships, the suicidal patient reflects a much greater effect in interpersonal relating which transcends the present illness and is apparent throughout the history of his relationships prior to the depressive illness. It is reiterated that emphasis should be placed on the clustering of factors for the more accurate identification of the potential suicide. Thus, depressed patients who succeed in maintaining distance, negating help or maintaining a consistently paranoid stance should be considered potential suicides. Pathological dependency may have the same distancing and isolating effect, as in the case of paranoia. This may explain the study of Farberow, et al.[93] showing "close intense relationships between suicidal patients and significant others."

The response of the significant other to suicidal communications may also be useful in the assessment of risk. It has been observed that their own defenses of naivete, fear, rejection, and denial often interfere with their responses to the patients' suicidal threats.[27] Shneidman[60] more specifically states that the behavior of the significant other, especially the wife, can be either life-saving or "suicidogenic." He states that "a spouse who is hostile, independent, competitive or nonsupportive appears to doom her husband to a suicidal outcome, whereas a wife who is helpful, emotionally supportive, and actively ancillary seems to save the man who might otherwise have killed himself." Conscious and unconscious death wishes on the part of the significant other, repression, anger and anxiety all tend to make it extremely difficult for family members to consider suicide of a relative as a possibility.[40,62,96,155-160] It has been stated that in every suicidal act there is an ambivalent desire to be rescued. However, often a potential rescuer may overlook or fail to respond to a suicidal communication, disregarding it "because of his own hostility or lack of ego strength and libidinal resources."[161]

It can be concluded that chronic personality features which appear to be related to lethal suicidal behavior are: 1) paranoid traits, 2) impaired interpersonal coping, 3) suicidal communication, 4) symbiotic dependency needs with reliance

on external controls, 5) rigid thinking, 6) externalized anger, 7) intermittent loss of control, and 8) chaotic energy levels with stimulus-seeking behavior. While the above more enduring characteristics associated with chronic high risk are of value, the clinician faced with the possibility of imminent suicide must also recognize acute behavioral changes as a signal for instituting life-saving therapeutic intervention.

ACUTE PRE-LETHAL FACTORS

It is difficult at best to take the stance with a patient or his family or friends that suicide may be imminent. Since there is no way of proving the point without losing a life, the clinician is in a position of having to rely strictly on his own experience or knowledge in making this crucial and oftentimes disputed judgment. Many behavioral features preceding suicide may also be observed in more general emotional crises. However, when these traits are noted in the presence of depressive symptomatology or psychodynamics characteristic of depression (failure, loss, etc.), the possibility of suicide should be carefully considered.

Clinicians, regardless of orientation will recognize several different types of suicidal patterns.[30,59,78] It is our purpose to identify certain acute behavioral and situational factors found with the greatest frequency in the seriously attempted and completed suicide (See Table II):

1) One of the best known and most reliable indicators of impending suicide can be obtained by direct repeated inquiry into the patient's suicidal thoughts.[9,30,127,162-165] Anxiety, self-accusation, weeping, moaning, or averted gaze can indicate suicidal intent. A response of anger has also been observed to be a potentially dangerous warning sign.[3] Many patients will admit to a suicidal plan. Experience has shown that the more detailed this plan and the more lethal the method contemplated, the greater the likelihood of suicide.[2,21,40,65,85,166,167] It has been observed in some cases that the absence of feelings of relief and gratitude toward those intervening to thwart the attempt, and the instead expressions of disappointment and anger can be suggestive of lethal intent.[3] Patients who use suicidal threats to control or dominate the behavior of others tend more often to regard suicide in vague terms.

In general, impulsive suicidal attempts tend to be less serious. A possible exception may occur in the case of the psychopathic personality or acutely schizophrenic individual. In this case, other behavioral features may be of help in the prevention of impulsive suicide. Potential warning signs include: a) a feeling of entrapment, b) psychological defenses such as denial crumbling when challeneged, c) a resultant sudden onset of intense depression.

2) A most important clinical feature noted in the seriously suicidal patient is that of abrupt clinical change. Various clinicians have commented on a chance for either better or worse, occurring rather suddenly, in patients just prior to suicide.[5,26,41,94,168-174] This may take the form of change from a rather anxious or agitated depression to a sudden mood of calm or peacefulness. Sudden onset of increased activity, life interest, or even euphoria, in a patient with a retarded depression has often been observed to precede suicide. "Paradoxical improvement" may be noted when there is a return of the energy to act and the ability to make decisions, yet prior to the return of a normal mood and hopeful outlook. This period between apparent improvement and the return of hope is often seen in depressed patients responding to antidepressant drugs.[109]

Relating to this point in his study of 881 suicides, Capstick[41] found that, of previously depressed patients, 35 percent appeared normal just prior to suicide. This supports the observation that the return of normal ego-functions may actually increase the risk of suicide. It is therefore a potentially dangerous error to accept uncritically improvement based on external appearances without careful inquiry into the patient's mood-state. A possible explanation for this seemingly incongru-

ous finding of a state of calm before suicide may be related to the observations of Spiegel and Neuringer[131] who found that genuine suicide notes were less explicit in suicidal intent and displayed fewer suicidal symptoms.

It was concluded that these individuals may have succeeded in overcoming the dread of death. Lester[175] found that adolescent suicidal students fear death less than control subjects. However, Lester's suicidal subjects were more concerned with the manipulative aspects of death threats. Piotrowski[89] found infrequent references to death in Rorschach protocols within one year of suicide. Therefore, the state of calm often reported in patients just prior to suicide may be correlated with their having finally overcome the state of dread related to suicidal impulses and having reached a "final solution" to a feeling of entrapment and doom. Perhaps related to this decreasing fear of death, Neuringer and Lettieri[92] have recently observed that when the high-risk individual feels self-destructive, there is a reversal of normal life attitudes, in which death is perceived as extremely positive, and life as extremely negative. However, because all of the subjects in their study were white females, the authors caution about generalizing their results until further replication can be done on other groups of patients.

A sudden unexplained exacerbation of a patient's clinical state may also herald a serious suicide attempt.[39,96,102,127] This decline may be marked by an increase in anxiety and depression related to the failure of previously functioning psychological defenses.

3) Failure of psychological defenses often precedes suicide.[26,30,88,129,176] It has been suggested that suicide occurs more frequently when obsessive symptomatology abates in the course of a depressive illness.[100] However, since these findings were based on a study of patients' records, rather than actual clinical observations, further investigation of this point seems warranted.

It has been clinically observed that a failure of paranoid defenses may occur just prior to suicide. The hatred and blame which the patient has successfully projected to the outside is suddenly attributed to himself.[106,109] Another extremely dangerous period seems to be when the paranoid patient begins projecting his suicidal impulses onto significant others (usually spouse).[177] Such patients may also fear that they are to be killed by others. Laboratory observations relating to alterations in 17-OHCS excretion in depressed patients prior to suicide also support the inference of an increased likelihood of suicide in a patient who shows evidence of failing defenses.[178]

4) Litman[130] has pointed out that mental regression, disorganization, and ego-splitting-pathologic processes which allow a portion of the ego to initiate action disregarding and threatening the survival of the self may occur just prior to suicide. The emphasis on mental regression stresses the importance of restoring the operation of basic ego functions in suicidal patients whether these be distorted by psychosis, hysterical dissociation, alcohol, fitlike or tantrum behavior, drug intoxication, or impulsive aggressive outbursts. Some type of ego-splitting, dissociation or regression is thus hypothesized as necessary to overcome the dread of death in order to initiate a true suicidal behavior.[21,58,126,129,162,177,179-184] A study by Trautman,[126] based on interviews with 93 Puerto Rican patients just prior to serious suicide attempts, illustrates the sudden mental regression which can occur just prior to suicide. Typical subjective descriptions include: "I suddenly lost control of myself"; "I suddenly lost my mind"; "My mind went blank"; "I didn't realize what I was doing"; etc. Trautman further observes that these patients report having completely lost their fear of death during this period, and comments that the first sign of alert mental functioning was the return of the dread of death. However, since Trautman's sample was focused solely on Puerto Ricans and was predominantly female (76 percent), it is difficult to generalize his findings to other cultural groups. A

history of abrupt loss of control and outbursts of physical violence in patients with other high-risk features may be useful indicators of imminent suicide.

5) It has frequently been observed that suicide may occur when a patient finds himself confronted by an outlook of utter hopelessness.[30,102,144,185-193] Clinicians should note carefully patients with dry-eyed sobbing, or quiet and undramatic suffering, who manifest symptoms of retardation or agitation.[3] Intensive clinical observations of patients prior to suicide have suggested that these patients are actually in a state of "delusional hopelessness." This goes beyond the usual feeling of hopelessness articulated by many depressive patients and amounts to a fixed belief that hope is, in fact, forever lost and nothing will ever help the situation.[109] In making the difficult distinction between delusional hopelessness and manipulative demands of the patient manifesting anger, one must look for true hopelessness regarding future change and total absence of bargaining responses. Lester[1] has concluded, however, that there is, as yet, only empirical evidence to support the view that suicidal individuals lack hope. This discrepancy can perhaps be explained on the basis that the experimental situation with its emphasis on objective tests may fail to reach into the underlying state of a patient's hopelessness.

6) Recent evidence of decreasing ability of a suicidal patient to project far into the future, which is related to the patient's sense of hope, may be of clinical value in the assessment of acute suicidal potential.[30,86,102,128,132,188,191,194-196] However, one must be careful to relying too heavily on future projection, since a patient who is believed to be making plans for the future may still be suicidal. Sometimes, such future arrangements serve the purpose of allaying the suspicions of relatives until the time when the patient is "ready" to die.[3] Recent development of a time-perspective measure by Yufit[197] may be of value in accurately evaluating the future orientation of the high-risk suicidal individual.

7) Observations of interpersonal distance may arise acutely in the patient progressing toward suicide. Three related features have been noted immediately preceding serious suicidal behavior.

A. Communication to Significant Other Only—Serious suicidal patients frequently made vague suicidal communications only to significant intimates, generally spouses, while not expressing this intent to others, e.g., their therapists, prior to suicide.[68,75,135]

B. Attempted Outer-directed Change—Patients frequently displayed specific behavior patterns or changes requested by their significant other and are interpreted as improvement just prior to their suicidal attempts.[135]

C. Death as Intent—Patients surviving near-lethal attempts subsequently stated their unequivocal wish to die. However, these patients frequently stopped talking about death or suicide for a brief period of time just prior to a serious suicide attempt.[135]

8) Clinicians have emphasized different elements of dream material as important in warning of a potential suicide. Mintz[183] and Raphling[198] have independently observed that manifest dream content centering around death, violence and suicidal themes signals the possibility of an impending suicide. Rahpling states, however, that the presence of these themes did not seem to be related to the lethality of patients' suicide attempts. Gutheil[80] states that symbolic scenes of death, with overtones of peacefulness, or dreams in which the patient meets his death with an exciting or even euphoric feeling,[179] are more predictive of actual suicide and thus may serve the function of mediating the acceptance of death or decrease in dread. Although Saul and Curtis[199] refer to dreams of falling, their cases apparently refer to suicidal urges rather than to suicidal outcome. Litman[73] has found that dreams with manifest content referring to symbols of separation often indicate immobilization in the significant other of the

TABLE II

ACUTE PRE-LETHAL FACTORS

1. Specific suicidal plan
2. Abrupt clinical change
3. Decreasing fear of death concomitant with an increasingly positive attitude toward death
4. Failure of psychological defenses
5. Mental regression
6. Delusional hopelessness
7. Loss of future perspective
8. Sudden decline of interpersonal relating, with help negation
9. Dreams of symbolic peaceful scenes of dying where death is looked upon as exciting or euphoric

potential suicide. From this section it can be concluded that the following features may indicate an imminent suicide: 1) abrupt clinical change, 2) failure of psychological defenses, 3) delusional hopelessness; 4) specific suicidal plan, 5) sudden decline of interpersonal relating, 6) mental regression, 7) loss of future perspective, 8) decreasing fear of death concomitant with an increasingly positive attitude toward death, and 9) dreams of symbolic peaceful scenes of dying where death is looked forward to as exciting or euphoric.

SITUATIONAL PRECURSORS OF SUICIDE

Probably the most commonly understood instances of increased suicidal risk in the depressed patient are situations associated with separation or loss[22,30,47,78,113,139,200-209] (Table III). The loss does not necessarily have to be the final loss or death of a loved one as Freud[115] emphasized, but may be simply a temporary loss to the patient who is in a depressive crisis.[78] Bolin, et al.[67] noted the importance of loss among his 27 suicidal cases and emphasized that in addition to real losses, threatened or imagined losses, over a six-month period, were closely correlated with the occurrence of suicide. He stresses that the summation of recent losses (spouse, home, job, hospital discharge, temporary separation from therapist, money, love, etc.) depends exclusively on the perception of the patient. Thus, to a depressed patient the anticipated loss of a significant relationship may be a more frequent observation prior to suicide than actual loss.[109,134,139,200]

Other situations of high suicide risk representative of loss include the Anniversary syndrome,[21,59,129,210-213] and the phenomenon of reactivation[213] often observed around the time of hospital discharge. In some cases the patient takes no overt recognition of the approaching anniversary date of the deceased. In other cases, a potential warning is a steady deepening of depression, climaxing in suicide on the anniversary date.[210] Moss and Hamilton[213] refer to this "reactivation" of suicidal impulses which they see as a non-regressive, working-through process which often occurs at the point when a formerly depressed suicidal patient begins to return to his previous functions during a period of improvement. This reactivation is supported by the observation that suicide frequently occurs in hospitalized patients when they begin going home on trial passes or just prior to the anticipation of hospital discharge when, due to the patients' "apparent" improvement, there is often a generally shared optimistic prognosis by staff.[3,30,40,215] This is probably related to some evidence that at least 50 percent of suicides in former inpatients will occur within six to twelve months after hospital discharge.[216-219] Indeed, there is clinical support for the contention that the first three months following hospital discharge can be especially critical.[67,113,220] Similarly, the termination of outpatient psychotherapy can be an extremely dangerous period in terms of sui-

cidal risk in certain individuals: 1) A severely schizoid patient who has been seen in long-term individual psychotherapy; 2) a patient who terminates therapy with expressions of ambivalence over an unresolved dependency-autonomy crisis, earmarked by depression and hopelessness, and 3) a patient who has no close relationship with any significant other, outside of therapy and his family.[221]

Another situation which may be associated with suicide could be termed "failure situation."[60,90,173,201,206] The identification of this situation with loss occurs because it often develops at about the time of hospital discharge when a patient is trying to regain or attain higher levels of function, such as successful engagement of a job or a return to college work. The phenomenon of reactivation mentioned above is often seen in this situation when patients try to meet higher expectations of themselves or others, as is common in the case of the schizophrenic patient with depressive features.

An overly optimistic staff pushing a recently recovered depressed patient beyond his own level of confidence may be shocked to find suicide the outcome. This occurrence may be explained by the intense anxiety generated when the magnitude of expected performance exceeds the amount of hope that the goal can be achieved, as has been formulated by Stotland.[222] In line with this, many therapists, even experienced ones, contribute unwittingly to the probability of serious suicide attempts in their patients by engaging in the following ineffective therapeutic procedures: 1) "Externalizing the superego" by reflecting the patient's self-hatred, leading to hopelessness, agitation, and suicide. 2) Interrupting crucial autistic defenses, without whose previous wish-fulfilling fantasies, the patient is confronted with an unbearable and depressing reality. 3) Developing a "symbiotic transference" of an extremely primitive nature, which is then broken off.[223]

The presence of real or perceived physical illness may be significant in the assessment of suicidal risk.[5,23,30,91,224-229]

TABLE III

SITUATIONAL PRECURSORS OF SUICIDE

1. Threatened or actual loss of relationship
2. Failure situation
3. Real or perceived physical illness

Dorpat, et al.[229] have reported in their series that, compared to the general population, 70 percent of the suicides occurred in the presence of poor physical health, depending on age. Illnesses that Dorpat et al. found to have a high association with the occurrence of suicide were rheumatoid arthritis, peptic ulcer, and hypertension. Sanborn, et al.[230] have recently found an association between separation or loss, acute dermatologic stress-related disease, depression and actual suicide. Similarly, a study by Farberow, et al.[231] of suicide among patients with malignant neoplasms revealed that high suicidal potential was found especially among older men, with cancer of the throat and younger men with Hodgkin's disease or leukemia, or persons of any age with cancer concomitant with heightened stress, severe anxiety, and low tolerance for pain. Bolin's study[67] of suicide among patients on hospital leave found physical health to be one of the most discriminating high-risk factors for both sexes, and, surprisingly, independent of age.

Various investigators have pointed out that the hypochondriasis of the patients who ultimately commit suicide is usually of a delusional type and that more often than not, one organ or system is the subject of concern.[36,40,62,85,106] It is rarely the widespread shifting hypochondriasis of the psychoneurotic patient. Yet, there is disagreement as to the relationship of a general hypochondriasis and suicide, some authors seeing a positive association[116,210] and some seeing hypochondriasis as a defense against suicide.[12] It does appear clear however, that fixed delusional hypochondriasis, especially that involving one organ, suggests an increased suicidal risk.

In malignant or incurable illness, two critical suicidal periods seem to be those of: a) uncertainty while diagnosis and prognosis are still at issue and, b) shock

following the first realization of the upheavals and suffering, actual or fantasized, that are to follow.[3] Paradoxically, however, a serious physical handicap such as blindness may serve as a protection against suicide—the suicidal danger is after restoration of sight, rather than when the vision is lost. The sudden realization that the depression has its source in the patient's own personality rather than in the blindness itself, makes suicide a plausible choice.[232] Depressed patients who fear that as the result of surgery, there will be important facial or body disfigurement, impairment of sexual image or loss of sexual potency may be high suicidal risks.[39] Finally, severe dyspnea coupled with severe emotional disorder has been observed to occur 24 hours prior to actual suicides.[26] Lester[1] has recently concluded that the association between illness and suicide may be a weak one. Perhaps this discrepancy can be explained by the clinical observation that perceived physical illnesses may be more meaningful and panic-provoking to the depressed individual than the presence of actual illness. Leonard[82] has classified three personality types prone to suicide and the conditions under which acute risk occurs: The "trigger" for suicide in the Dependent Dissatisfied patient (DD) seems to occur when, in his chronic complaining, there is an urgency and a desperation that he can no longer find a new victim or resource on whom to play his push-pull games. For the Satisfied Symbiotic type (SS) the "trigger" for suicide appears to be the loss or threat of loss of the closely loved one. This will precipitate "an unusual disturbance in an otherwise well-behaved person—tears, agitation, depression, self-condemnation, sleeplessness and physical complaints." The SS is unable to blame anyone but himself and cannot be pushed to express anger toward the loved one. Unlike the DD, there is no great anger expressed either just prior to or through the suicidal act. The contrast between the SS patients' usual attempts to please and the acute onset of the above symptoms is a dramatic indicator that suicide may be imminent. The "trigger" in the unaccepting type (US) appears to occur at the time when there is an unexpected change in his life's situation producing loss of prestige and independence which he is powerless to prevent, such as a business failure or severe illness.[82] Related to this, Miller[233] in her analysis of 25 suicides, has observed that suicide may occur in the unaccepting type when this individual begins to doubt his ability to fulfill his rigid heroic self-image. Often just prior to suicide this person has been putting his affairs in order or "planning" for the future as though he might not be present. Suicide in these "strong" individuals may be very unexpected since they manifest few symptoms severe enough to warn of their intent.[82]

THE DYNAMICS OF SUICIDE IN YOUTH

The recently observed increase in suicide in children, adolescents and young adults directs our attention to the behavioral features which might help identify suicidal youths.[234] It is doubtful whether children under the age of nine actually intend to die, because of an inability of a child to completely conceptualize death. Paradoxically, a child or even an adolescent, may wish to kill himself but may not wish to die.[22,235] Seiden and Sobel and Margolis have reported repetitive self-poisoning by children, usually beginning at age two, reflecting pathological family conditions. Many of the pre-suicidal signs related to children tend to be highly nonspecific and may be useful only when considered in association with more specific acute clinical signs.

Frederick[234] lists the following acute behavioral clues preceding suicidal behavior in potentially self-destructive youth: "1) Adolescents or youngsters contemplating suicide are more likely to communicate with a peer than with their parents. 2) They may give away a prized possession with the comment that they will not be needing it any more. 3) The individual is apt to be more morose and

isolated than usual. 4) While insomnia, worry and anorexia often appear, the youngster may not have all the classical signs of depression. Some investigators, however, have concluded that more classical depressive symptoms are common in children and adolescents who are suicidal.[158,173] 5) The girl often turns to a boyfriend for support and he "rejects" her because he is not capable of satisfying her demands. 6) Recent involvement with drugs occurs. 7) There is something in the youngster's behavior suggesting that he wants to get even with his parents. 8) Frequently the girl may believe she is pregnant. The risk of completed suicide during pregnancy, however, approaches zero. The risk increases in the period following pregnancy when: 9) There is evidence of a postpartum depressive reaction with psychosis; the mother's personality is characterized by instability and marital stress; and there is rejection and loss of support by the father or the mother's family.[236]

The danger of completed suicide is substantial among adolescents and young adults in the acute phases of a schizophrenic illness, particularly if it is accompanied by an acute paranoid reaction or acute homosexual guilt or panic.[3,30,39,56,94,237,238] In acute suicidal panic states, one serious warning is a paradoxical absence of suffering in the facial expression of these patients.[3]

While anhedonia has been associated with suicide in schizophrenia,[55,59,210] the following factors have been recognized by Warnes[55] to indicate that schizophrenic suicide may be imminent: "1) Prolonged and increased hopeless awareness of psychopathology, 2) feelings of inner disintegration, 3) agitation and hostility, and 4) severe persecutory anxiety and restlessness." Auditory hallucinations which order suicide or threaten life may be regarded as serious indicators of possible suicide.[3,22,44]

Friedman[239] has emphasized that suicides or attempts are frequently connected with tremendous guilt feelings resulting from conflicts over masturbation. It was theorized that masturbation may be a defense against homosexual wishes and that suicide often occurs when masturbation has ceased. Thus, an almost obsessive concern with the struggle to avoid masturbation may be a sign of increased suicidal risk. Moreover, Friedman considers the combination of parricidal wishes and incestuous drives as pathognomic for adolescent suicides.[169] Resnik[240] has described a syndrome of "erotized repetitive hangings" in which adolescent males engage in masturbation titrated to increasing self-manipulative neck pressure. When opportunities for detection decrease and when self-rescue is made progressively more difficult, a true suicidal act may be brought about in order to escape from overwhelming feelings of depression and anxiety. Clinicians, therefore, are alerted to question any unusual neck bruises on their young male patients. Guilt over sexual acting-out may be a major factor precipitating female adolescent suicides.[22] The literature suggests that school and social failures, with resultant isolation, may be trigger factors in the individual with severe sexual conflicts. It has been stated that only the student who abandons all hope of love throws his life away.[239]

Parrish[241] has listed symptoms occurring in 25 college suicides in order of their frequency: "despondency, futility, lack of interest in school work, a feeling of tenseness around people, insomnia, suicidal communications, fatigue and malaise without apparent organic cause, feelings of inadequacy or unworthiness, and brooding over death of a loved one." Studies on college students do not appear to emphasize sexual conflicts in connection with suicidal behavior with the frequency that is noted in younger adolescent patients.

NEW DIRECTIONS IN SUICIDE RISK ASSESSMENT

In recent years there have been a number of additional findings relating to suicide which have pointed to new areas

useful in the understanding and evaluation of suicidal potential.

Psychobiological Research

Special emphasis on the occurrence of changes in the state of psychological defenses and the experience with states of extreme anxiety suggest that biological measures may be of use in the identification of certain high-risk periods in suicidal patients. The possible association between the metabolic excretion of 17-hydroxycorticosteroid (17-OHCS) and suicidal potential was first suggested by Bunney and Fawcett.[178,242] The contention, that unusual elevation of 17-OHCS may act as a possible biochemical index of suicide potential, has been observed in a recent clinical and physiological review of one case.[243] More recently, Fawcett and Kerste[244] found that in a series of 55 depressed inpatients, five patients (two suicides and three serious attempts) ranked much higher than the total group in 17-OHCS excretion during the first two weeks of admission. In this study 17-OHCS levels were found to correlate more positively with subsequent suicidal behavior over several weeks to two months, than clinical assessment on admission. Levy and Hansen[245] could not replicate elevations of 17-OHCS excretion in two cases of suicide, but their results were complicated by the presence of medications which may have interfered with the measurement of high levels. Krieger[246] reported some correlation between elevated plasma 17-OHCS on admission and suicide on a long-term (9-to-29-month) follow-up. However, in these reports it is emphasized that low steroid values certainly never could be used as assurance of low suicide risk in patients otherwise believed to be in a period of high suicidal potential.[178, 242,244] Yet of particular interest (for additional research) is the finding by Struce, et al.[247] on the association between paroxysmal abnormal electroencephalograms, 17-OHCS, and suicidal behavior.

A related paper showed increased cholesterol content, a precursor of the adrenal stress hormone, in a small group of patients who died by suicide, in comparison with a similar group who died by sudden accidental deaths.[248] Shaw, et al.[249] presented the analysis of brain electrolytes in depressive and alcoholic patients who committed suicide and found "an increase in water content and low concentrations of sodium in the depressed group, while the alcoholic group were characterized by remarkably high water and sodium content and by low concentrations of potassium chloride." These findings are of interest since it is known that the adrenal stress hormones reflected by 17-OHCS levels have profound effects on body electrolytes. Another finding relating to steroid hormones in suicide is the interesting association between suicidal behavior and distress as found in women during the early menstrual, premenstrual and midluteal phase of the menstrual cycle.[250,251] A recent review by Wetzel and McClure,[252] however, has found a considerable number of methodological problems and inconsistencies in most available studies on suicide and the menstrual cycle. Thus, further studies of the interaction between altered ratios of hormone levels seem to be indicated in psychobiological studies of suicide.

Other studies pointing to the possibility of biological changes related to suicidal behavior are those of Shaw, et al.,[253] who found low 5-hydroxytryptamine (5-HT) levels and of Bourne, et al.,[254] who found low levels of 5-hydroxyindoleacetic acid, the major metabolite of serotonin, in certain areas of the brains of patients who committed suicide. In this regard it is of interest that studies in the rat have shown that injections of steroid hormones can result in a drop of 5-HT levels in the brain suggesting a possible interrelationship between steroid levels, salt-retention and biogenic amine metabolism in the depressed suicidal patient.[255,256]

Psychophysiology Research

Twenty years ago, E. C. Voth suggested a relationship between suicide and the

limited capacity to experience autokinesis, a phenomenon which is best described as apparent movement of a stationary pinpoint of light in a totally dark room. Voth employed the psychoanalytic construct of ego-closeness (a relatively unwavering investment of attention in the external field in people who experience little or no autokinesis) and stated that this should be observable in patients making suicidal attempts and completed suicides.[88] Significantly, Voth found that those who only attempt suicide are more ego-distant than those who commit suicide. The 19 cases of subsequent suicide in one clinical group showed a trend toward less autokinetic movement than did the attempt-group, although this difference fell just below statistical significance. Voth concluded that the risk of suicide progressively increases as autokinesis decreases. This finding supports clinical observations concerning rigid thinking in suicidal patients. Specifically, Neuringer and Lettieri[92] have suggested that rigid and dichotomous thinking can be viewed as an essential cognitive trait in the high-risk suicidal individual. However, schizophrenics, even with high autokinetic scores, do sometimes impulsively kill themselves.[88] The autokinesis measure also may be related to the perception of external locus of control found to correlate positively with high suicidal risk.[76,226]

Another psychophysiological area of some promise is suggested by the findings of a decrease in autonomic reactivity as measured by Galvanic skin response (GSR) to the affective meaning of suicide during word association experiments. Spiegel[257] found this measure to discriminate between suicide attempters and threateners. This decrease in autonomic reactivity may be related to a reduced level of dread of death described as occurring prior to suicide.[131]

Status of Psychological Testing

Many attempts have been made to evaluate suicide potential through the use of psychological measures. Thus far, however, no such measures have been found to predict suicide successfully, largely due to problems in test design.[258,259] Nevertheless, the development of a psychological instrument which will reflect suicidal potential has attracted the interest of several investigators.

Promising Signs

No matter what card it is perceived on, nor whether it is observed just once or many times, the color shading response to the Rorschach test was found with greater frequency in groups of suicides and attempts than in controls, by Appelbaum and Holtzman.[260] However, the later finding that 49 percent of the controls tend also to give color shading responses detracts from the predictive validity of this sign.[147] Previous suicidal scales have been helpful in the detection of the chronic suicidal individual.[50,95,261,262] Beck's new suicidal ideation scale, however, shows promise in detecting the urgency and acute thinking of the lethal suicidal individual.[167] Piotrowski[89] developed a suicidal scale based on his research with 60 suicidal patients and has shown that 12 Rorschach signs correlate positively with high suicidal risk.

Time Perspective

In a study of depressed inpatients, outpatients, and control subjects, an instrument measuring a patient's capacity to invest himself in the future showed that seriously suicidal patients may be discriminated on the basis of less future time perspective.[197]

Outlook for Testing

While it is unlikely that any one test will be constructed to measure all the relevant dimensions which require evaluation in the assessment of the suicidal patient, efforts seem to point to the utilization of a task-oriented measurement which will include a range of relevant variables.[259] The test development in this area may elaborate useful concepts which can be used in the clinical setting even if a comprehensive battery is not realized.

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report measures in relation to a suicide attempt. *J Psychiatr Res 8:127, 1971*

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ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Cardiology

Kessler H, Liebson PR, Mattenheimer H, Adams EC, Jr: *Myoglobinuria in the diagnosis of acute myocardial infarction. Am J Cardiol 35:148, 1975*

This study was accomplished to assess the specificity of hemagglutination-inhibition (HI) determination of urinary myoglobin (Mb) in myocardial infarction (MI). Mb was isolated from monkey or human tissue. Mb sheep erythrocyte conjugate was prepared, and antisera to Mb was produced in rabbits and goats. This test detects concentrations as low as 0.6 μg/ml. Aliquots of urine from 39 patients (pts.) with suspected MI were studied from HI. Serial serum enzyme and electrocardiogram were used for definitive diagnosis of MI. Of 24 patients with documented MI, 15 had at least one positive HI study (63 percent). Ten of 13 patients with MI (77 percent) who were studied within 24 hours of the acute event had positive reactions in the infarct group decreased sharply after 24 hours, giving results of 38 percent, 31 percent and 25 percent positivity on days two, three and four, respectively. None of the 15 patients without infarction had positive tests. This test appears to be a sensitive, specific, non-invasive technique in rapid screening of patients with possible acute myocardial infarction during the early stage of symptomatology.

Liebson PR, Mann LI, Evans MI, Duchin S, Arditi L: *Cardiac performance during pregnancy: Serial evaluation using external systolic time intervals. Am J Obstet Gynecol 122:1, 1975*

Indirect systolic time indices were used to evaluate serial changes in left ventricular function during pregnancy in 13 normal patients and five patients with compensated cardiac conditions. Changes in both groups tended to parallel each other. In both groups, serial LVET index decreased, and PEP/LVET ratio increased. Q-S_2 index decreased in the normal group in midpregnancy and was inconsistent in the cardiac group. In the immediate postpartum period, LVET index returned to baseline values but PEP and PEP/LVET remained increased in both groups. Results of multiple comparison tests demonstrated that the largest (or smallest) value of each systolic time interval during pregnancy was observed in either the periods of the weeks 28 to 34 or 38. No significant differences were noted in intergroup comparison between the normal and cardiac groups. These data suggest that consistent changes in cardiac function may be determined during pregnancy by the noninvasive technique of indirect systolic time-interval evaluation. These changes may reflect a change in left ventricular contractility, preload or afterload. The differences may persist even in the immediate postpartum period when cardiac output and heart rate have decreased to normal, suggesting residual changes in intrinsic left ventricular function. In patients with compensated heart disease, indirect tests of left ventricular function generally reflect the changes in normal pregnant women.

Dermatology

Epstein WL, Baer H, Dawson CR, Khurana RG: *Poison oak hyposensitization: evaluation of purified urushiol. Arch Dermatol 109:356, 1974*

More than 500 volunteers were patch tested with dilutions of purified poison oak urushiol in acetone. Of this group, 69 subjects participated in three experiments that conclusively

showed that large amounts of urushiol taken over a period of three or more months produced measurable hyposensitization, as detected by repeated patch testing. No systemic toxicity was observed, but pruritus ani was common, and occasional skin eruptions were seen when too much drug was given. These cutaneous symptoms and signs disappeared once the subject had become hyposensitized.

Diagnostic Radiology

Huckman MS, Neer D, Norton T: *Convexity meningioma presenting angiographically as "pseudo-subdural hematoma": report of a case. Neurochirurgia 17:66, 1974*

A patient with the clinical and angiographic picture of a subdural hematoma was found at surgery to have a meningioma. Meningioma as well as the other causes of "pseudo-subdural hematoma" should be considered when evaluating patients with head trauma since the indications for surgery and the operative approach in other entities differ from those for subdural hematoma.

Endocrinology and Metabolism

Lebbin D, Ryan WG, Schwartz TB: *Outpatient treatment of Paget's disease of bone with mithramycin. Ann Intern Med 81:635, 1974*

Mithramycin is a cytotoxic antibiotic that causes hypocalcemia and diminishes the elevated osteoclastic activity characteristic of Paget's disease. Previous inpatient studies have shown that this drug causes striking clinical and biochemical improvement in patients with severe widespread disease. To simplify therapy and lessen toxicity, 13 patients with this form of Paget's disease were treated for 1 to 14 months with either of two different outpatient regimens. During therapy the elevation in the serum alkaline phosphatase decreased by 40 percent to 99 percent, and all patients reported symptomatic improvement. Manifestations of toxicity were mild and transient.

Genetics and Human Development

Sachdeva S, Smith GF, Justice P: *Case report. An unusual chromosomal segregation in a family with a translocation between chromosomes 3 and 12. J Med Genet 11:303, 1974*

A family is reported in which two infants were born with different types of congenital abnormalities. Chromosome studies on one of the infants showed a partial trisomy of the short arms of a No. 3 chromosome. A family study showed many balanced translocation carriers who had extra chromosomal material on the long arms of a No. 12 chromosome.

Smith GF: *The lymphocyte in culture: historic perspective. The National Foundation Original Article Series 9:5, 1973*

The lymphocyte may be the key to unlocking some of man's biologic mysteries. From the initial observations of Denis Burkitt on lymphomas in African children, there has been a series of important experiments and observations which have markedly increased our knowledge about lymphocytes in long-term culture.

Baker PJ, Rubin LG, Lint TF, McLeod BC, Gewurz H: *Binding of the complement intermediate C$\overline{56}$ to zymosan in acute phase human sera. Clin Exp Immunol 20:113, 1975*

C$\overline{56}$ is known to appear in the fluid phase when zymosan is incubated at 37°C with certain acute phase 'reactor' sera. In the present study, C$\overline{56}$ was detected bound to the zymosan particle prior to its appearance free in solution. In reactor sera C$\overline{56}$ was formed and released with kinetics similar to that of the generation and decay of a C$\overline{56}$-binding site formed when zymosan was incubated with normal serum. Bound and fluid phase C$\overline{56}$ was detected only in reactor sera, and was generated only by agents known preferentially to activate the properdin pathway. Elution of C$\overline{56}$ from zymosan in hypertonic salt solutions proved to be a convenient step in the partial purification of large amounts of this haemolytically active bimolecular complex.

Baker PJ, Lint TF, McLeod BC, Behrends CL, Gewurz H: *Studies on the inhibition of C$\overline{56}$-induced lysis (reactive lysis) VI. Modulation of C$\overline{56}$-induced lysis by polyanions and polycations. J Immunol 114:554, 1975*

Multiple polyanions and polycations were tested for their ability to influence formation of EC$\overline{567}$ from C$\overline{56}$, C7, and sheep erythrocytes. Six of 11 polyanions tested, including polyanethol sulfonate, heparin, and dextran sulfate, inhibited this reaction. By contrast, polycations (five of seven tested), including polybrene, protamine, and polynithine, potentiated formation of EC$\overline{567}$. The inhibition was similar to that previously described for anionic serum factors termed C$\overline{567}$-INH, while the potentiation seemed to involve neutralization of serum C$\overline{567}$-INH. Thus, this step of the complement attack mechanism seems amenable to modulation by certain polyelectrolytes, and may thereby be susceptible to pharmacologic manipulation.

McLeod B, Baker P, Behrends C, Gewurz H: *Studies of the inhibition of C$\overline{56}$-initiated lysis (reactive lysis) IV. Antagonism of the inhibitory activity C$\overline{567}$-INH by poly-l-lysine. Immunology 28:379, 1975*

The stable intermediate complex C$\overline{56}$ can initiate the lysis (reactive lysis) of unsensitized erythrocytes (E) by the membrane attack mechanism of complement. Certain serum constituents designated C$\overline{567}$-INH inhibit reactive lysis by preventing the C$\overline{567}$ complex, once formed, from attaching to a membrane surface. It is shown here that microgram quantities of poly-l-lysine (PLL), a synthetic polycation of molecular weight 180,000, can reverse the effects of C$\overline{567}$-INH, and thereby potentiate formation of EC$\overline{567}$ by erythrocytes, C$\overline{56}$ and C7 in whole serum. Erythrocytes exposed to PLL in a preincubation step did not show either increased susceptibility to C$\overline{567}$ or resistance to C$\overline{567}$-INH, and reversal of C$\overline{567}$-INH by given amounts of PLL was not diminished as cell concentrations were greatly increased, indicating that the effect of PLL was predominantly directed against fluid phase rather than against erythrocyte membrane substrates. The effects of PLL and C$\overline{567}$-INH were quantitatively reciprocal. Thus, PLL-induced potentiation of C$\overline{56}$-induced lysis is a solute effect which seems to involve direct neutralization of naturally occurring serum inhibitors of the C$\overline{567}$ trimolecular complex of complement. The use of PLL thus provides a suitable antagonist for C$\overline{567}$-INH in reaction mixtures, and allows evaluation of the role of C$\overline{567}$ and C$\overline{567}$-INH in a variety of situations involving C-mediated lysis.

McLeod B, Baker P, Gewurz H: *Studies on the inhibition of C$\overline{56}$ initiated lysis (reactive lysis) I. Description of the phenomenon and methods of assay. Immunology 26:1145, 1974*

Reactive lysis refers to the lysis of unsensitized cells by late acting complement components (C5-C9) acting independently of early components. The existence in human

serum of an activity which inhibits reactive lysis had been inferred previously from certain morphological features of the reactive lysis of red cells suspended in agarose gels, and from the difficulty with which reactive lysis proceeds in whole serum. We have found this activity, which we abbreviate 'INH-RL,' in all human sera tested, and in the serum of several experimental animals. We have developed two methods of detection of INH-RL in gels, and have established a sensitive, quantitative assay for INH-RL in solution, which has been adapted to the measurement of INH-RL in serum. We have examined the effect of INH-RL on the measurement of $C\overline{56}$ and C7 by reactive lysis in gels. Inhibition of distal complement components in primary lysis of EAC142 by INH-RL was detectable but was markedly less potent than inhibition of reactive lysis. INH-RL is distinct from other known complement inhibitors, and thus represents a newly appreciated regulatory mechanism in the complement cascade. It may have importance in quenching the indiscriminate haemolytic activity which can be generated from complement in the fluid phase, especially by stimulation of the alternative pathway.

McLeod BC, Baker P, Gewurz H: *Studies on the inhibition of $C\overline{56}$-initiated lysis (reactive lysis). II. $C\overline{567}$-INH—an inhibitor of the $C\overline{567}$ trimolecular complex of complement. Int Arch Allergy Appl Immunol 47:632, 1974*

We have recently described an activity in serum designated "INH-RL" which inhibits $C\overline{56}$-initiated lysis (reactive lysis) of sheep red cells and which may have a role in prevention of damage to host tissue during complement activation. This activity did not prevent either formation of $C\overline{567}$ from $C\overline{56}$ and C7 or consumption of C8 and C9 by these complexes. Amounts of active material which prevented $C\overline{56}$-initiated lysis in dilute GPC-EDTA did not prevent lysis of $EC\overline{567}$ in the same reagent; however, formation of $EC\overline{567}$ from E, $C\overline{56}$, and C7 was inhibited. This inhibition could not be overcome by an excess of either $C\overline{56}$ or C7 alone, but it was overcome by an excess of both $C\overline{56}$ and C7. Consumption of C8 and C9 by mixtures of $C\overline{56}$ and C7 was not inhibited by amounts of active material which would prevent EC567 formation. Thus, these experiments show that this activity has its predominate effect upon preventing formation of $EC\overline{567}$ by E and $C\overline{567}$. This activity is distinct from previously described inhibitors of complement. Despite the observations that is activity is shared by several serum proteins, its site of action is discrete. Therefore, it is suggested that the designation $C\overline{567}$ inhibitor ($C\overline{567}$-INH) be used to describe this activity.

McLeod B, Baker P, Gewurz H: *Studies on the inhibition of $C\overline{56}$-initiated lysis (reactive lysis) III. Characterization of the inhibitory activity C567-INH and its mode of action. Immunology 28:133, 1975*

An activity in serum which inhibits reactive lysis has recently been shown to do so by preventing the attachment of $C\overline{567}$ complexes to cells, and hence has been designated $C\overline{567}$-INH. This report describes certain physicochemical characteristics of the inhibitory activity. It behaves as a heat-stable pseudoglobulin, soluble in 20 percent Na_2SO_4, and having $\alpha 1$ mobility on Pevikon block electrophoresis. It is excluded from CM cellulose at pH 6·0, RSC = 0·007 M, is retained by an XM-100 membrane and is heterogenous on Sephadex G-200, eluting in at least two peaks. The combined active materials from the Sephadex column elute from DE-52 in at least four peaks. The mechanism of action of material from each of these four peaks is shown to involve prevention of attachment of $C\overline{567}$ complexes to membranes, and this is shown to involve an effect on $C\overline{567}$ complexes in solution rather than an effect on the membrane. A less dramatic effect on the lysis of $EC\overline{567}$ by limited quantities of C8 and C9 can be demonstrated. Haemolytic studies using cell-bound $C\overline{567}$ suggest that the interaction of $C\overline{567}$-INH with $C\overline{567}$ involves a loose reversible association. It is therefore postulated that $C\overline{567}$-INH inhibits reactive lysis primarily by reversibly associating with the nascent $C\overline{567}$ complex in solution, increasing its bulk and decreasing its diffusion capacity so that it is unable to reach a cell membrane before its haemolytic potential decays.

McLeod B, Lint TF, Baker P, Behrends C, Gewurz H: *Studies on the inhibition of C56-initiated lysis (reactive lysis) v. The role of C567-INH in the regulation of complement-dependent haemolysis initiated by cobra venom factor. Immunology 28:741, 1975*

Activation of the alternative pathway of complement by a factor from cobra venom (CVF) can lead to lysis of unsensitized erythrcytes (E) of some species. In these studies we observed that alterations in CVF-induced lysis could be produced by manipulation of C567-INH, a naturally occurring inhibitory activity which acts on fluid phase C567 complexes. Venom lysis of sheep and guinea-pig E was markedly inhibited by serum fractions having C567-INH activity. Microgram quantities of poly-L-lysine (PLL), molecular weight 180,000, a polycation which is a functional antagonist to C567-INH in serum, potentiated CVF lysis of sheep and guinea-pig E, and permitted the lysis of human E, which are otherwise not susceptible to CVF lysis. The potentiation of venom lysis by PLL seemed not to be due to alterations in the target cell membrane; furthermore, it in turn was reversed by substances with C567-INH activity. This suggests that the generation of fluid phase C567 complexes contributes to the CVF-induced lysis of erythrocytes of these species, and that the haemolytic potential of fluid phase C567 generated during alternative pathway activation by this means is regulated by C567-INH.

Mortensen RF, Osmand AP, Gewurz H: *Effects of C-reactive protein on the lymphoid system. I. Binding to thymus-dependent lymphocytes and alteration of their functions. J Exp Med 141:821, 1975*

C-reactive protein (CRP) is an acute phase protein which shares with the immunoglobulins the ability to induce precipitation and agglutination reactions and activate the complement system. We report here that purified human CRP binds selectively to human T lymphocytes, inhibits their ability to form spontaneous rosettes with sheep erythrocytes and inhibits their response to allogeneic cells in mixed lymphocyte culture reactions; it fails to inhibit phytohemagglutinin- or concanavalin-A-induced mitogenesis. CRP does not bind to human B lymphocytes, nor does it alter the following B-cell functions: binding to activated complement components or the Fc protion of immunoglobulins, mediation of antibody-dependent cytotoxicity reactions or the ability of allogeneic cells to stimulate a mixed lypmhocyte culture reaction. Human CRP shows similar selective binding with murine T lymphocytes. It therefore seems that binding of CRP is a property of T lymphocytes or a subpopulation thereof, and can result in modulation of certain of the T-cell functional characteristics *in vitro*. We suggest that CRP may play a role in modulating T-cell functions during the inflammatory state.

Rent R, Ertel N, Eisenstein R, Gewurz H: *Complement activation by interaction of polyanions and polycations. I. Heparin-protamine induced consumption of complement. J Immunol 1:124, 1975*

Interactions of heparin and protamine in fresh human serum, in amounts far below those required for complement depletion by either agent alone, were found to induce virtually complete depletion of total hemolytic complement activity. This depletion was dependent on time, temperature, pH, divalent cations, and serum concentration. The predominant complement component hemolytic activity depleted was C1; under appropriate reaction conditions C4 and C2 were depleted as well. Equivalent amounts of heparin alone induced lesser but substantial depletion of C1, potentiation of C4 and C2, and minimal depletion of C3-9, whereas equivalent amounts of protamine had no effect upon complement component activities. We conclude that interaction of heparin with protamine, like interaction of antibody, with antigen, markedly enhances its ability to interact with the first component of complement and activate the classical complement pathway. It is suggested that complement activation by interactions between certain polyanions and polycations, like interactions between antigens and antibodies, may have a role in the initiation of inflammatory reactions.

Schutte M, DiCamelli R, Murphy P, Sadove M, Gewurz H: *C3 proactivator (C3PA) as an acute phase reactant. Clin Exp Immunol 18:256, 1974*

C3PA levels were measured sequentially in four patients prior to and following general anaesthesia and surgery, and in twenty patients with active inflammatory diseases. Elevated levels (+70 percent and +60 percent, respectively) were observed in both patient groups, indicating that C3PA is a potent acute phase reactant. Peak levels were seen four days following surgery, one to two days after the peak of the C-reactive protein response. C3PA, along with C1s, were the complement proteins which of those tested showed the greatest elevation during the acute phase response. We conclude that the serum C3PA level should be interpreted with consideration of its role as an acute phase reactant.

Siegel J, Rent R, Gewurz H: *Interactions of C-reactive protein with the complement system. I. Protamine-induced consumption of complement in acute phase sera. J Exp Med 3:647, 1974*

Protamine sulfate was found to consume large amounts of C selectively during preincubation with sera of individuals in the "acute phase." Marked depletion of C1, C4, and C2 with minimal, if any, depletion of C3-9, was observed. The consumption was time and temperature dependent, occurring most rapidly and extensively at 37°C, 0.10 M relative salt concentration and pH 7.5-8.0; it required calcium ions. It was mediated by a heat-stable nondialyzable factor which separated with C-reactive protein (CRP) during fractionation and purification, correlated with serum CRP levels, and, like other known reactivities of CRP, was inhibited by phosphoryl choline. Preparations of CRP purified either from serum or ascites resulted in consumption of large amounts of C1, C4, and C2 when preincubated with normal serum and protamine. We conclude that CRP is a potent activator of the C system at the level of C1, and that polycations such as protamine sulfate are substrates of CRP which can bring about this activation. It seems not unlikely that one role of CRP in health and disease involves its ability to interact with the C system.

Snyder A, Hand MR, Gewurz H: *A rapid pH stat assay for plasma prekallikrein and fluctuations in disease. Int Arch Allergy Appl Immunol 47:411, 1974*

We report a new pH stat adaptation for the assay of human plasma prekallikrein. Kaolin was used to activate this enzyme and the cleavage of TAMe was quantified by the continuous recording of H^+ released rather than by the widely used method of sampling to measure the amount of methanol released. Prekallikrein levels in 80 samples assayed by the two methods correlated well. The pH stat assay was found to be advantageous because of its simplicity, speed (15 to 30 min), accuracy and reduction of variables. Using these procedures, we confirmed the previously described reduction of prekallikrein in patients with bacterial infections and chronic liver disease, and detected lowered levels in patients with active systemic lupus erythematosus and normocomplementemic rheumatoid arthritis.

Microbiology

Falk L, Nigida S, Wolfe L, Deinhardt F: *Herpesvirus ateles:* in vitro *and* in vivo *studies. Proc Amer Assoc Cancer Res 15:55, 1974*

Herpesvirus ateles (HVA), isolated from peripheral lymphocytes of spider monkeys, was compared with Herpesvirus saimiri (HVS): 1) antigenically, 2) host range *in vitro* and 3) oncogenicity in marmoset and squirrel monkeys. HVS and HVA share common antigens as measured by immunofluorescent tests and antibodies reacting against both viruses were found in a majority of serum samples from spider, squirrel and woolly

monkeys. Growth of HVA and HVS *in vitro* is limited to cell cultures from simian species, but HVA failed to replicate in vero cells which are sensitive to HVS. Marmosets developed malignant lymphoma and died 17 to 28 days after infection with HVA; most developed antibodies to HVA, and virus was isolated from their peripheral lymphocytes by cocultivation methods. HVA caused no overt disease in squirrel monkeys; they became seropositive but virus was not recovered from their peripheral lymphocytes. A lymphoblastoid cell culture was established from tumor cells of one marmoset; a small percentage of the cells express HVA antigens and forms infectious centers when cultured on permissive cells. The cell line possesses T-cell properties: rosettes are formed with sheep erythrocytes but immunoglobulins are not produced. Marmoset lymphocytes have been transformed *in vitro* by cocultivation with this HVA-carrying, X-irradiated cell line. Thus far transformation with HVS by similar methods has been unsuccessful.

Falk L, Wright J, Wolfe L, Deinhardt F: *Herpesvirus ateles: Transformation* in vitro *of marmoset splenic lymphocytes. Int J Cancer 14:251, 1974*

Four lymphoblastoid cell cultures were established by transformation of marmoset splenic lymphocytes *in vitro* with Herpesvirus ateles (HVA). The association of HVA with each cell line was demonstrated by the recovery of HVA from the lymphoblastoid cells or from the culture fluids and by the demonstration of HVA antigens in a small percentage of cells of each lymphoblastoid culture. Intranuclear inclusion bodies were observed in three cultures examined but herpesvirus virions were observed in only one of three cell lines. The four cell cultures had properties of T-lymphocytes.

Falk LA, Nigida SM, Deinhardt F, Wolfe LG, Cooper RW, Hernandez-Camacho JI: *Herpesvirus ateles: properties of an oncogenic herpesvirus isolated from circulating lymphocytes of spider monkeys (ateles sp.) Int J Cancer 14:473, 1972*

Herpesviruses were isolated from lymphocytes from four of eighteen spider monkeys imported recently from Colombia, South America. Virus was isolated after circulating lymphocytes were co-cultivated with permissive cell cultures. Three isolates induced fatal malignant lymphomas in cotton-topped and white-lipped marmoset monkeys and one isolate which was also tested in squirrel monkeys caused no overt disease in this animal species. These virus isolates shared cross-reacting antigens with herpesvirus saimiri but possessed distinguishing biological properties.

Falk LA, Wolfe LG, Wright J, Deinhardt F: *Herpesvirus ateles: Transformation of marmoset lymphocytes* in vitro. *Abst Annual Meeting Amer Soc Microbiol, 1974*

Herpesvirus ateles (HVA), isolated in our laboratory from peripheral lymphocytes of spider *(Ateles sp.)* monkeys, is oncogenic for marmoset *(Saguinus sp.)* but not squirrel monkeys *(Saimiri sciuresis).* HVA and Herpesvirus saimiri share cross-reacting antigens as measured by immunofluorescence (FA) tests but exhibit different properties in cell cultures. An HVA-carrying lymphoblastoid cell line, 1022, was established from peripheral lymphocytes of a marmoset experimentally-infected with HVA. Peripheral lymphocytes of marmoset, squirrel and macaque monkeys and baboons and marmoset spleen lymphocytes were cocultivated with lethally X-irradiated 1022 cells. Five lymphoblastoid cell lines were established from lymphocytes obtained from spleens of five marmosets; none of the peripheral lymphocyte samples became transformed after 90 days cultivation. The cultivation period before transformation was evident ranged from 17 to 27 days. Cells of all five cultures formed infectious centers in permissive cells and small amounts of infectious HVA were recovered from culture fluids. HVA antigens were observed in a small number of cells after FA staining and herpesvirus virions were present in cells of several cultures. No membrane immunoglobulins were demonstrated but 40 to 80 percent of cells formed rosettes with sheep erythrocytes, indicating the cell lines were composed of T cells.

Peterson DA, Wolfe LG, Deinhardt F, Gajdusek DC, Gibbs CJ Jr: *Transmission of kuru and Creutzfeldt-Jakob disease to marmoset monkeys. Intervirology 2:14, 1973-4*

Two degenerative diseases of the central nervous system of man, kuru and Creutzfeldt-Jakob disease, were transmitted to marmoset monkeys. Kuru was passed serially, with decrease in the latent periods.

Svedmyr EA, Deinhardt F, Klein G: *Sensitivity of different target cells to the killing action of peripheral lymphocytes stimulated by autologous lymphoblastoid cell lines. Int J Cancer 13:891, 1974*

Peripheral lymphocytes obtained from two individuals with a previous history of infectious mononucleosis were exposed to mitomycin-treated cells of the autologous lymphoblastoid cell line (LCL) established during the acute phase of the disease. This resulted in a stimulation of DNA synthesis, comparable to or even exceeding a one-way MLC with allogeneic lymphocytes. The cytotoxic effect of the stimulated lymphocytes was tested by colony inhibition of ^{51}Cr release, against a large LCL panel, including the autologous line and allogeneic lines from patients with Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), infectious mononucleosis (IM), leukemia, myeloma, or normal donors. While the majority of the lines were highly sensitive to the killing action, three were relatively resistant. The same pattern of sensitivity was obtained with effector cells stimulated by autologous LCL derived from IM or BL patients. The majority of the target LCLs had B-cell characteristics and carried the EBV genome, but three cell lines that were devoid of the EBV genome, were also sensitive. These lines included two lymphoid cell lines, one of them a T-cell line, and a myeloma line. Fresh peripheral lymphocytes from normal donors or acute IM patients, PHA-induced blasts and blast cells from a case of acute myeloid leukemia were resistant.

Wright J, Falk LA, Deinhardt F: *Interferon production by simian lymphoblastoid cell lines. J Natl Cancer Inst 53:271, 1974*

Six lymphoblastoid cell cultures, established from tumors induced in cotton-topped and white-lipped marmosets with *Herpesvirus saimiri* (HVS) were studied for spontaneous interferon production over a period of 14 months. Five of six cell lines produced virus inhibitor at levels from 4 to 256 interferom U/ml. The cell lines were virus-producing when first established in culture, but production of infectious HVS, formation of infectious centers in permissive cell cultures, and expression of virus-specific antigens by the cells decreased progressively during 14 months' cultivation, with little or no change in levels of interferon production.

Nephrology

Bartlow BG, Oyama JH, Ing TS, Miller AW, Economou SG, Rennie IDB, Lewis EJ: *Glomerular ultrastructural abnormalities in a patient with mixed IgG-IgM essential cryoglobulinemic glomerulonephritis. Nephron 14:309, 1975*

Distinct glomerular ultrastructural lesions in the form of nodular subepithelial and intramembranous, as well as discrete and band-like subendothelial deposits are described in a patient suffering from mixed IgG-IgM essential cryoglobulinemic glomerulonephritis. It is suggested that the deposits may represent immune complexes which are believed to play a central role in the pathogenesis of the glomerulonephritis.

Armbruster KFW, Rahn AC, Ing TS, Halper IS, Oyama JH, Klawans HL: *Amantadine Toxicity in a patient with renal insufficiency. Nephron Basel, S. Karger 13:183, 1974*

In a patient with chronic renal failure and maintained with regular hemodialysis, the development of neuropsychiatric untoward effects during amantadine therapy was associated with an inordinately elevated plasma level of the drug. It is recommended that in the face of renal inadequacy, adjustment of the dosage of the drug should be done in accordance with the degree of renal dysfunction.

Ing TS, Ashbach DL, Kanter A, Oyama JH, Armbruster KFW, Merkel FK: *Fluid removal with negative-pressure hydrostatic ultra-filtration using a partial vacuum. Nephron 14:451, 1975*

Removal of excessive fluid with a negative-pressure hydrostatic ultrafiltration technique using a partial vacuum was performed successfully in eight patients. The technique appears to be a satisfactory means of fluid removal.

Rosenberg JC, Arnstein AR, Ing TS, Pierce JM Jr, Rosenberg B, Silva Y, Walt AJ: *Calculi complicating a renal transplant. Am J Surg 129:326, 1975*

Four months after a cadaver kidney transplant, kidney stones were found in the renal allograft. Three major predisposing causes of nephrolithiasis were found in the patient, including hyperparathyroidism, renal tubular acidosis, and urinary tract infection. Hypercalcemia was corrected by parathyroidectomy. During the subsequent three years there was no enlargement of the renal stones, and adequate kidney function was maintained. Renal tubular acidosis was not severe and seemed to be related to chronic rejection. Urinary tract infection was readily corrected with antibiotics and did not recur after the immediate post-transplant period. Surgical therapy for nephrolithiasis involving a kidney allograft was deferred since urinary flow was not obstructed. This course of management is recommended for use in patients with calculi complicating renal transplantation.

Neurological Surgery

Bartlow B, Penn RD: *A carotid cavernous fistula presenting as a posterior fossa mass. J Neurosurg 42:585, 1975*

A 38-year-old man developed trigeminal neuralgia and ipsilateral hearing loss fourteen years following a severe head injury. Arteriography demonstrated a carotid-cavernous sinus fistula draining into the posterior fossa and producing a large venous mass. This is the first reported case of a posterior fossa mass caused by such a fistula.

Selby RC: *Neurosurgical aspects of leprosy. Surg Neurol 2:165, 1974*

The classification, clinical manifestations, and indications and methods of neurosurgical diagnosis and treatment of leprosy have been reviewed. The role of surgery for the management of nerve abscess, some entrapment syndromes and methods to establish diagnosis have been indicated. Further improvements in medical management of the disease and its complications will lessen the therapeutic role of surgery. Increasing knowledge of the pathogenesis of leprosy and erythema nodosum leprosum (ENL) probably will shed light on the mechanisms involved in the production of other neuropathies, particularly those associated with collagen diseases.

Selby R, Pereira N: *Intracranial neoplasms in Malaysia. Int Surg 58:536, 1973*

An analysis of 357 cranial and intracranial neoplasms among Malaysians revealed a relative incidence of the major types of tumors similar to that reported by others. Among

the gliomas glioblastoma multiforme and medulloblastoma were less frequent and ependymoma more frequent than described in similar studies. Cerebellar and other primary intracranial sarcomas were considerably more common. Further studies of racial and environmental factors as they relate to the incidence of tumors may be aided by similar analyses from other countries.

Ver Brugghen A: *Intervertebral infections following surgery. Surg Neurol 2:426, 1974*

The author, who has had an extensive surgical experience with herniated intervertebral discs, has never encountered a postoperative intervertebral infection. He has described his methods of operating and set forth why it appears to him that he has had no postoperative infections of this type.

Obstetrics and Gynecology

Campanella R, Wolff JR: *Emotional reaction to sterilization. Obstet Gynecol 45:331, 1975*

Ninety-four patients sterilized by tubal occlusion were interviewed at the time of hospitalization for surgery and followed at scheduled intervals for a two-year period. There were no serious problems, medical or psychologic, and all but three patients were pleased and satisfied at the end of two years. There were no technical failures. Psychosomatic symptoms do develop. They are more prominent and persist longer in the younger age group. Patients having these symptoms are those who had difficulty with contraceptive technics, have a limited understanding of the sterilization procedure, and continue to question its permanency. The need for preoperative evaluation and counseling is emphasized.

Oncology

Levin AC, Schauf V, Wolter J, Deinhardt F: *Correlation of cellular immunity with clinical course in breast cancer. Proc Am Assoc Cancer Res 15:55, 1974*

The cellular immunity of patients with breast cancer was correlated with the clinical course of the disease. Twenty-four women with Stage II and III breast cancer and twenty healthy controls were studied for lymphocytotoxicity *in vitro* against allogeneic, cultured breast cancer cells and control fibroblasts, using a micro-^{51}Cr release assay. A significant difference was found between the cytotoxicity of patients with slowly progressive disease (38 percent) and both those with rapidly progressive disease (17 percent) and normal healthy controls (19 percent). The degree of lymphocytotoxicity correlated directly with the length of the disease-free interval between mastectomy and the first recurrence or metastasis.

Organ Transplantation

Couser WG, Lewis EJ: *Laboratory suggestion: A method for preservation of immunofluorescence in renal tissue. Am J Clin Pathol 61:873, 1974*

A method which utilizes a modified semipermanent mounting medium and cold storage to preserve sections of renal tissue stained for immunofluorescence microscopy is described. Results demonstrate that tissue prepared and stored as described undergoes no alteration in the pattern or intensity of immunofluorescent staining or in morphology for

periods exceeding two years. The use of this method and standardized immunofluorescent reagents facilitates centralized interpretation of renal immunopathology in collaborative studies of renal disease.

Merkel FK, Jonasson O, Bergan JJ: *Procurement of cadaver donor organs: evisceration technique. Transplant Proc, 4:585, 1972; and* In *Clinical Transplantation, D. M. Hume and F. T. Rapaport, Editors, New York, Grune & Stratton, 1972, pp. 159-163*

An evisceration technique was developed for cadaver kidney procurement and is used for patients sustaining cerebral death and being maintained with cardiorespiratory support. Homeostasis is achieved by use of concentrated albumin, judicious use of crystalloid pressors, and diuretics. The stomach, spleen, duodenum, jejunum and large intestine are rapidly excised providing exceptional views of the kidneys. They are then excised *en bloc* and immediately cooled and prepared for preservation. This technique avoids renal or vascular trauma, facilitates the use of kidneys with multiple renal arteries, and allows for ease of cannulation via the aorta.

Merkel FK, Seim SK, Armbruster K, Firlit CF, King LR: *Thrombosis of a perfused cadaver kidney. Urology 4:709, 1974*

A cadaver kidney was excised and preserved by pulsatile perfusion at another medical center. Only one artery was identified. During the transplant procedure at our institution, a large inferior pole vessel was encountered and both vessels were anastomosed. Several days after transplant the perfused portion of the kidney thrombosed and the non-perfused segment sustained life. The patient later died of aspergillus mycotic aneurysm of the brain and both renal artery anastomoses were found to be technically without flaw, thus suggesting the possibility of perfusion injury.

Pediatrics

Page El E, Grossman HJ: *Neurologic appraisal in learning disorders. Pediatr Clin North Am 20:599, 1973*

The neurologic examination must be followed by an interpretation of findings and in many instances a final diagnosis based on neurologic findings alone will not be possible. Grouping together signs and symptoms into identifiable syndromes, when possible, and relating them to other diagnostic information, can assist in future planning for the child.

This paper is an attempt to provide the pediatrician with some general principles and practical guides to the office evaluation of children with a suspected learning disability.

Pharmacology

Bolton WK, Spargo BA, Lewis EJ: *Chronic autologous immune complex glomerulopathy: effect of cyproheptadine. J Lab Clin Med 83:695, 1974*

The histologic and clinical features of experimental autologous immune complex glomerulopathy in rats indicate that glomerular damage is due to the deposition of antigen-antibody complexes along the glomerular basement membrane and is analogous to chronic mebranous glomerulopathy in man. As the deposition of antigen-antibody complexes within blood vessel walls may be retarded by the action of vasoactive amine antagonists, the present study was undertaken in order to determine the effect of cyproheptadine, an agent with antihistamine and antiserotonin properties, upon the development of proteinuria in appropriately immunized rats. Two regimens of cyproheptadine were studied: animals in Group IA received 0.5 mg per kilogram of the drug every 8 hours for an eight-week period. Animals in Group IIA received 1.5 mg per kilogram in a single daily injection. Immunized, untreated littermates served as controls (Groups

IB and IIB). Proteinuria (> 10 mg protein per day) began to appear among animals in the immunized untreated groups four to five weeks after immunization and affected 82 percent of the rats in Control Group IB, and 96 percent of the rats in Control Group IIB eight weeks after immunization. Proteinuria did not appear among the treated animals in Group IA until the sixth week after immunization, and the prevalence of proteinuria in this group was lower than that of their controls (Group IB) at each weekly interval. In addition, the amount of protein excreted by the treated animals in Group IA that became proteinuric was significantly less than that of their controls in Group IB. Similarly, animals in Group IIA had a significantly lower prevalence of proteinuria compared to their controls (Group IIB) at each weekly interval. The quantity of proteinuria in the proteinuric animals of Group IIA was also lower than that of their controls, but the latter result was not statistically significant. Immunofluorescence microscopic examination of biopsies taken six weeks after immunization suggested that treated animals had a decreased deposition of immune complexes along the glomerular basement membrane compared to untreated controls. We conclude that the vasoactive amine antagonist, cyproheptadine, delays the onset of proteinuria and diminishes the degree of proteinuria in this experimental model of chronic immune-complex mediated glomerular damage.

Psychiatry

Cavanaugh JL Jr: *Career decisions in the early postresidency years. Am J Psychiatry 132:277, 1975*

The author notes that the end of the postresidency military obligation necessitates earlier career decision making for the senior psychiatric resident. This problem is seen to be exacerbated by the eclectic nature of most psychiatric training. Issues for residents include the problem of role models and the "senior resident syndrome," the decision of whether to maintain their university affiliation, the role of private practice, questions of clinical maturity, etc. The author believes that programs must be developed to systematically and objectively present to residents the data that now exist regarding multiple career options.

Surgery

Johnson DR, Straus A: *A technic for neonatal thymectomy in marmoset monkeys. Lab Anim Sci 24:343, 1974*

A technic was developed for thymectomy of neonatal marmosets under diethyl ether anesthesia. Thymectomy was performed within 24 hours of birth. Oral antibiotic therapy was continued seven days postoperatively. Preliminary experiments indicated that the degree of impairment of cell-mediated immunity is not as great in neonatally thymectomized marmosets as in mice.

Southwick HW: *Head and neck cancer: Cancer of the larynx: Surgical management. Lippincott, Proc Seventh National Cancer Conf Los Angeles, California, pp. 155-158, 1973*

The role of surgery in the treatment of cancer of the larynx is dominant for the more advanced lesions (T_3 and T_4), the radiation failures, and in the treatment of patients with clinical evidence of cervical node metastases. Preoperative radiation therapy diminished the incidence of local recurrence in the neck and should precede the neck dissection. The low-dose regime seems to be as effective as the high-dose and is economically more feasible. Conservation surgery is indicated in certain highly selected clinical situations.

RUSH-PRESBYTERIAN-ST. LUKE'S

MEDICAL BULLETIN

VOL. 14, NO. 3 JULY 1975

The Stein-Leventhal Syndrome

A SYMPOSIUM

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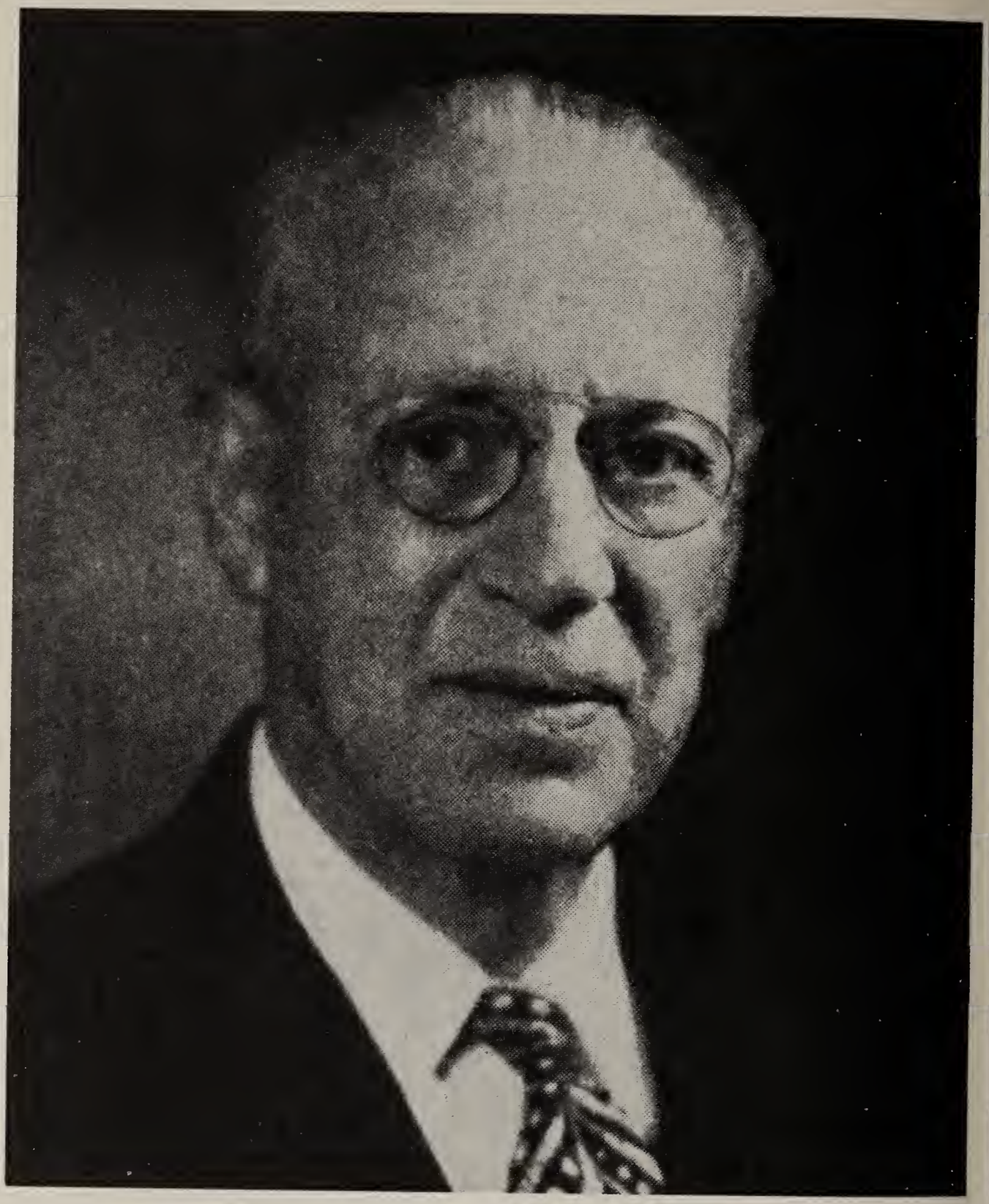

DR. IRVING F. STEIN

Irving Freiler Stein was born in Chicago, September 19, 1887. He received his B.S. degree from the University of Michigan in 1910 and his M.D. from Rush Medical College in 1912. After completing his internship at the Michael Reese Hospital, he joined its Department of Obstetrics and Gynecology and rose to the rank of senior attending. He is associate professor emeritus in the Department of Obstetrics and Gynecology at Northwestern University Medical School and serves as consultant to Highland Park Hospital.

DR. MICHAEL L. LEVENTHAL

The late Dr. Michael Leventhal (1901-1971) was also a graduate of Rush Medical College (1924), having received a B.S. degree from the University of Chicago in 1922. He was affiliated with Michael Reese Hospital throughout his distinguished career, which also included service with the United States Army in Africa, Sicily, and Italy.

Editor's Note

On April 18, 1975, Rush-Presbyterian-St. Luke's Medical Center presented, under the auspices of its Department of Obstetrics and Gynecology, a symposium marking the fortieth anniversary of the first exposition of the Stein-Leventhal syndrome, as well as of Dr. Irving Stein's service as President of the Chicago Gynecological Society.

At a luncheon honoring Doctor Stein, which preceded the symposium, Dr. James A. Campbell, President of the medical center, presented an appreciative resolution. Dr. Stein responded, and there were further remarks by Dr. J. P. Greenhill.

Dr. George D. Wilbanks, Chairman of Obstetrics and Gynecology, has written a foreword to the papers that are presented. The Medical Bulletin is pleased to carry the report of this symposium.

Evan M. Barton
Editor

Resolution

(Presented by James A. Campbell, M.D., President of Rush-Presbyterian-St. Luke's Medical Center, at the Stein-Leventhal Symposium, April 18, 1975, at Chicago, Illinois)

WHEREAS, for more than sixty years of professional dedication, Dr. Irving F. Stein has harmonized the demands of patient care with the responsibilities of teaching and research, and

WHEREAS, through his devotion to his patients and his determination to understand the mechanisms of fertility and sterility, he has helped to bring a richness of new life to countless families, and

WHEREAS, he has at all times presented for his fellow Rush graduates, his fellow physicians, and his fellow citizens the model of personal and professional integrity,

THEREFORE, on this, the fortieth anniversary of the first presentation of the Stein-Leventhal syndrome, we at Rush-Presbyterian-St. Luke's Medical Center do hereby express our profound admiration and warm appreciation for his achievements and for his example.

Dr. J. P. Greenhill then spoke as follows:

Remarks

J. P. GREENHILL

Ladies and Gentlemen:

I want to thank you for the honor of inviting me here today. I have known Irving Stein for more than 50 years, most likely longer than anyone in the audience.

I knew Irv before he wrote his first paper on "Polycystic Ovaries".

For many years Stein had been interested in "Pelvic Pneumoperitoneum" later called "Gynecography." In 1949 Irving F. Stein, Melvin Cohen and Ralph Elson wrote an important article, entitled "*Results of Bilateral Ovarian Wedge Resection in 47 Cases of Sterility: Twenty Year End Results; 75 Cases of Bilateral Polycystic Ovaries* (Am. J. Obst. Gynec. 58:267, 1949).

The syndrome frequently described by Stein and also by Leventhal quickly became recognized all over the world, and within a few years it was given the eponym "Stein-Leventhal Syndrome," which is still being used deservedly.

Naturally, and perhaps also because I have been the editor of the Year Books of Obstetrics and Gynecology for almost 50 years, I have read every article published by Stein and by others in the United States and abroad, dealing with the Stein-Leventhal subjects.

The papers which you will hear delivered by outstanding authorities indicate how much progress has been made in the Stein-Leventhal syndrome and similar conditions concerning the etiology, symptomatology, diagnosis and treatment of polycystic ovaries.

As a result of Stein and Leventhal's contribution thousands of babies are alive today who would not be here without the treatment advocated by Stein.

There is no question in anyone's mind that Irving Stein well deserves today's great honor and homage. He is fortunate in being able to live to see the rich fruition of his very important study—a privilege granted to few people.

Irving Stein has not only made a most outstanding contribution to medicine but also he has always been a perfect gentleman, most helpful to his colleagues and students and to his thousands of obstetric and gynecologic patients who worship him. To add to all this he is a true humanitarian. I want to wish him a much longer life in good health.

J. P. Greenhill, M.D., is Senior Attending Physician on the staffs of Michael Reese Hospital and Cook County Hospital, Chicago, Illinois

Response

DR. IRVING F. STEIN

Mr. Chairman, Fellow Alumni, Ladies and Gentlemen:

I am deeply grateful for the recognition and honor afforded me by the Rush Alumni, Rush College, the Chicago Gynecological Society and by Drs. Campbell and Greenhill for my part in describing a clinical syndrome almost half a century ago. This symptom complex which occurs in but a very small proportion of women with the problem of infertility has proved to be one of the rare instances of reversibility of sterility by means of a simple surgical procedure. In recent years this has also been accomplished by medical means in some instances.

The practice of obstetrics and gynecology has been a happy experience, but most gratifying to me was the result of an accidental discovery of a cure, while seeking the cause of secondary amenorreha (and hence, sterility), in a few young married women and some single adolescents.

My interest in the subject of fertility and sterility was aroused before 1920 and the investigation in this field was shared by my associates—late Michael Leventhal, Melvin Cohen, Bernard Kaye and the late Paula Bennett whose collaboration contributed importantly to our studies and publications.

In addition to achieving pregnancy by wedge resection of polycystic ovaries in previously sterile women, of significant benefit was the incentive afforded the endocrinologist to investigate the linkage between the clinical interruption of ovulatory function and the unusual ovarian changes that were found. Also challenging was an explanation of restoration of function by the surgical procedure employed.

Acceptance of the syndrome has been widespread but by no means universal. Some physicians even deny the existence of a definite syndrome. However, we are convinced of its validity and over a period of 35 years performed wedge resections of the polycystic ovaries in 108 women. There were 180 pregnancies with 144 live births reported (1964).

Without presenting further details, these results having long been published, may I conclude by thanking you for the gracious manner in which you have acknowledged my efforts to contribute something of interest and value in our special field of medical practice.

Foreword to the Symposium

In this symposium we honor Dr. Stein on the fortieth anniversary of his report, "Amenorrhea Associated with Bilateral Polycystic Ovaries," a syndrome known and recognized around the world.

This symposium is a melding of the old and the new Rush Medical College. Dr. Irving Stein, Sr. and Dr. Michael Leventhal exemplify the solid training that characterized the physicians of the old Rush. These gentlemen were not only physicians expert in providing service to their patients, but by their astute observation, developed hypotheses for basic research.

In the study of women who were infertile and amenorrheic, hysterosalpingography was performed. Enlarged ovaries were noted in some. This led the authors to develop the technique of pneumoroentgenography, which determined the presence of enlarged ovaries. Patients whose ovaries were biopsied started menstruating, and several of them later became pregnant. Originally performed as a biopsy this wedge resection later became a therapeutic measure. Thus, astute clinicians and investigative minds developed a recognizable syndrome.

Now to the "middle Rush." Although not a Rush graduate, Dr. Frederick Hofmeister trained in Chicago and at Presbyterian Hospital under Doctors DeLee and Allen. He developed vaginal surgery as another approach to the treatment of these women.

Currently, Drs. Julian Archie and Gretajo Northrop, "new Rush," are using laparoscopy and advanced radiologic techniques to obtain samples for detailed chemical analysis. And again, we have at Rush a clinical and scientific basis to interpret these new sophisticated developments in pharmacology, physiology, and endocrinology.

I want to thank the Chicago Gynecological Society for joining with us, and Dr. John R. Wolff for organizing this seminar.

GEORGE D. WILBANKS, M.D.
Guest Editor

George D. Wilbanks, M.D. is the John M. Simpson Chairman of the Department of Obstetrics and Gynecology at Rush-Presbertian-St. Luke's Medical Center, Chicago, Illinois

FURTHER EXPERIENCES COMPARING OVARIAN AND ADRENAL VEIN ANDROGENS WITH LAPAROSCOPIC FINDINGS IN HIRSUTE WOMEN: BIOCHEMICAL AND LAPAROSCOPIC FINDINGS

Gretajo Northrop
Julian Archie
Suresh K. Patel

The greatest problem encountered by the hirsute woman is finding a physician who will address himself to her problem. Routinely, after obtaining a urinary 17-ketosteroid analysis concentration and possibly a plasma testosterone level, the patient is comforted with an authoritative statement that she has constitutional or physiologic hirsutism. A lady who is growing a beard is not concerned about medical names; she only wants it stopped. It is frequently very frightening for her to learn of "normal values," because this means to her no treatment because she has no disease. Frequently (about 50 percent of the time) irregular menses and obesity accompany symptoms in these patients, and this, of course, leads to investigation of the thyroid gland. Even in the presence of normal thyroid function, the patient may be placed on thyroid medication. Fortunately, almost always, a very low dose is used, and the patient escapes unharmed by this medication. At this point depending upon the physician's background, the patient is usually placed on glucocorticoids, birth control pills or Clomid.®*

Thus, we may have a problem in which the lady is in excellent biological health except for clinical hirsutism, and not always can we prove that she has excess androgen by measuring a pooled sample of body fluids of one type. In other words, if we measure androgens in an antecubital vein sample or in a composite urine sample, we may not be able to show that this lady has an increase in androgen. However, most investigators who have studied hirsute ladies in detail report that they do secrete an increased amount of androgen necessary to document why excess is said to be present.

It is obvious that either excessive substrate is present which can be metabolized to the male hormones, or that the enzyme systems required to metabolize the male hormones to estrogens are compromised, or both (Table I). In either case, excessive testosterone or androstenedione, or both, or other steroids may result. I realize that biochemistry is not the forte of everyone here, but if you will bear with me I think I can make this a little bit clearer (Fig. 1). The point I would like make is that almost all androgens as well

TABLE I
EXCESSIVE ANDROGEN MAY BE PRODUCED IN TWO WAYS

1) Excessive Substrate Production
2) Limited enzyme for conversion of: Androstenedione to Estrone Testosterone to Estradiol

Gretajo Northrop, M.D., Ph.D., Assistant Attending Physician, Presbyterian-St. Luke's Hospital; Assistant Professor of Medicine and Obstetrics/Gynecology, Rush Medical College

Julian Archie, M.D., M.P.H., Assistant Attending Physician, Presbyterian-St. Luke's Hospital; Assistant Professor of Obstetrics/Gynecolc Rush Medical College

Sures Patel, M.D., Senior Attending Physician, Presbyterian-St. Luke's Hospital; Assistant Professor of Diagnotic Radiology, Rush Medical College, Chicago, Illinois

*Clomiphene citrate (Merrell-National)

as other steroids start out as cholesterol. Cholesterol is metabolized to pregnenolone, which is the basic steroid structure we are all interested in. Obviously there are many places where this molecule may go. It may go to make the commonly known mineral corticoid, aldosterone, or it may be metabolized into cortisol. Another pathway is to androstenedione or testosterone and then metabolized into estrogens. It is obvious that relative blockade of cortisol or aldosterone production may lead to an excess of androgen precursor which may be converted to physiologically active androgen. It is also obvious that partial blockade of enzymes required for converting androgens to estrogens may cause excessive androgen levels.

There are three possible areas where androgen production may take place in the non-pregnant woman who has no tumor. It may take place either in the adrenal or in the gonad, and since the female gonad is the ovary, these are the two major sites. In addition, there are multiple places in the body that can convert steroid intermediates to androstenedione or testosterone or both. Among the places of conversion are the liver and fat cells. So when we view a lady clinically, we need to know: (1) does she have an abnormal amount of androgen originating in her ovary, because we might want to treat that in one way? or (2) does she have an abnormal amount of androgen originating from the adrenal or from one of those two places, and is it being converted to an androgen at a peripheral site? To help resolve this dilemma, we routinely obtain blood samples for hormone analysis from the efferent adrenal and ovarian vessels.

I would like to discuss 53 hirsute patients which have been studied in collaboration with Dr. Patel and Dr. Archie during recent months.

Data on urinary 17-ketosteroid excre-

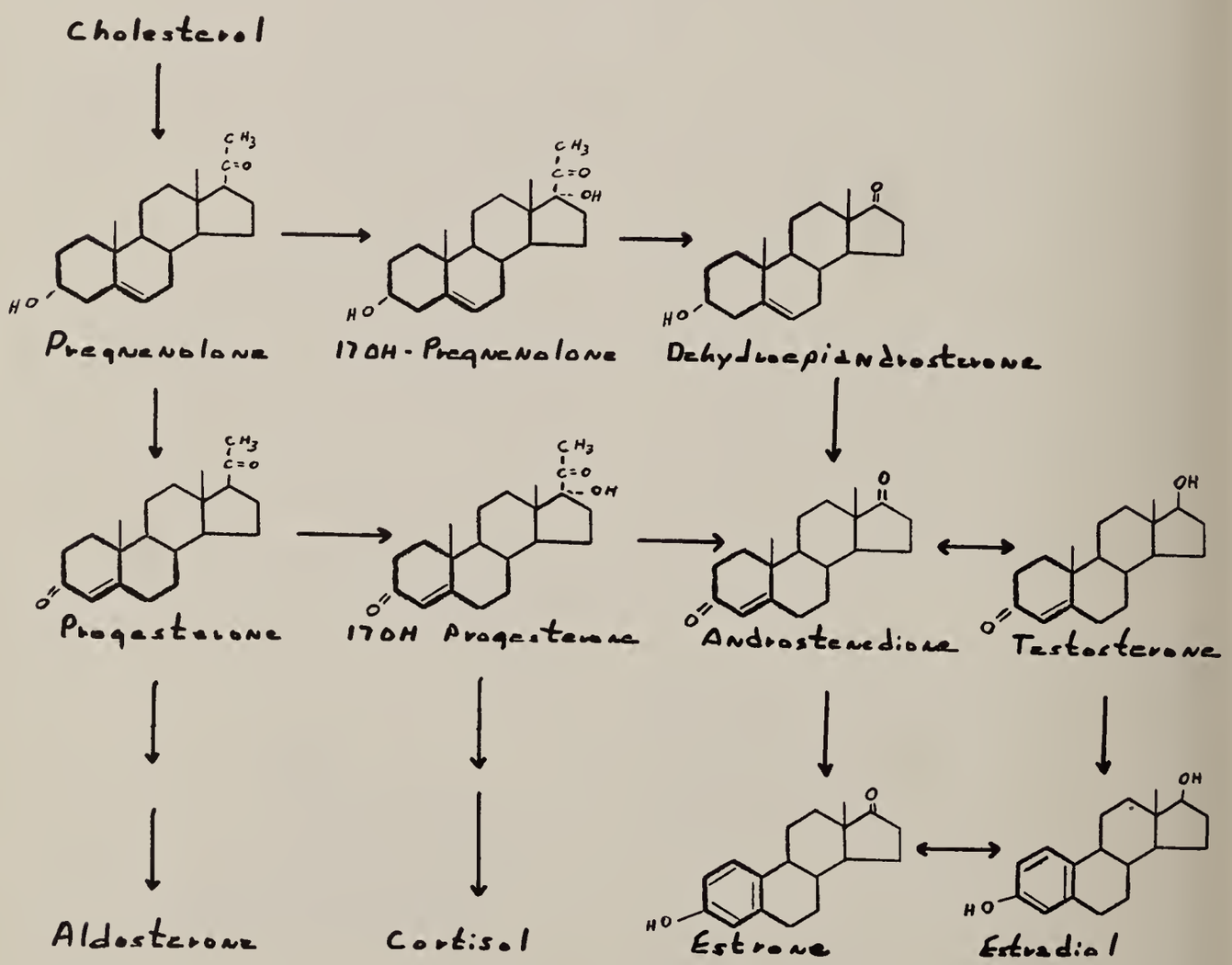

Fig. 1—A metabolic pathway is represented for androgen and estrogen synthesis.

tion are presented in Table II. It is obvious that the majority of the patients, (40) had normal 17-ketosteroid values. Thirteen patients had some excess, but it is interesting that the excess is not large in any of them. Clearly, this is not a good screening, and we should not run the test and then tell the patient that she is not making excessive amounts of testosterone. Most patients who are secreting excessive amounts of testoesterone will not be excreting excesive amounts of 17-ketosteroids into their urine.

Thyroid function was within normal limits, as indicated by either a normal PBI or T_4 by Murphy-Pattee, in all hirsute women studied.

I'd like to talk for a minute about gonadotropin levels in these ladies. There are many reports in the present literature in which the authors conclude that these ladies have elevated gonadotropin levels. They have high fluctuating levels of follicle stimulating hormone (FSH), and they do not have the luteninzing hormone (LH) pre-ovulatory spike. In all of these ladies tested, not one had a high level of gonadotropins with no LH spikes (Table III).

Plasma cortisol was evaluated in these women at 8:00 a.m. and at 4:00 p.m. It should be recalled that the daily ACTH spike in normal day-working people occurs between 1:00 and 3:00 a.m. and that high plasma cortisol should occur in the morning, with a drop of approximately 50 percent expected by late afternoon.

One of the earliest laboratory clues of Cushing's disease is the lack of diurnal variation, high values in the morning, and low values in the afternoon. Of interest here is that about half of these ladies do not show normal diurnal variation (Table IV).

TABLE II

BASELINE URINARY 17-KETOSTEROID EXCRETION IN 53 HIRSUTE WOMEN

Number of Patients	17-Ketosteroids mv/24 hours
19	3-8
21	8-12
13	12-17.1
normal female	3-12

TABLE IV

STUDIES ON DIURNAL VARIATION IN PLASMA CORTISOL IN 46 HIRSUTE WOMEN

Number of Patients	Plasma Cortisol Variation
21	Normal
25	Afternoon value less than 40% of Morning value

In Cushing's disease we say this is because of hypothalamic disease. I am quite interested in following these patients to see if this might be an indication of hypothalamic disease. However, there is nothing in the literature at this time, and we do not have additional information to tell you.

It is apparent, when considering the data (Table V) that peripheral plasma androgen levels do not correlate with clinical hirsutism. Forty-one of 50 clinically hirsute women had normal testosterone values, and 36 had normal androstendione

TABLE III

GONADOTROPIN LEVELS WERE IN THE NORMAL RANGE IN ALL HIRSUTE WOMEN STUDIED

Number of Patients	Hormone Studies (Normal)	Range of Values in Patients
42	FSH (2-50 mIU)	3-15
41	LH (5-100 mIU)	4-50
28	Urinary Gonadotropins (6-48 MU)	6-24

TABLE V
BASELINE PLASMA ANDROGEN CONCENTRATIONS IN 53 HIRSUTE WOMEN

Number of Hirsute Women	Peripheral Plasma Androgen	
	Testosterone	Androstenedione
Normal	41	36
Elevated	12	17
Female Normal in Ng%	20-60	90-280

levels. You may recall, only 13 women had elevated 17-ketosteroid levels. So, if we were to select the single best screening test performed on these 53 women we would select plasma androstenedione, as this is best correlated with clinical hirsutism of the tests described.

We have all heard today about gynecography. This was one of the early methods used to evaluate ovarian size. Fig. 2 is a picture of the sacrum (midline), the pubis, and here are the ovaries. This view was obtained, for the benefit of the younger people present, by putting air in the peritoneal cavity and then taking an x-ray. It is very obvious that the ovaries are enlarged. It is well demonstrated in the early studies, that the size of the normal ovary may not be greater than one-half the size of the uterus. This is a classic picture seen in a lady with polycystic ovaries of Stein-Leventhal syndrome. The patient was examined by three gynecologists who reported she had a normal pelvis. We previously used this test quite frequently, but we have discontinued us-

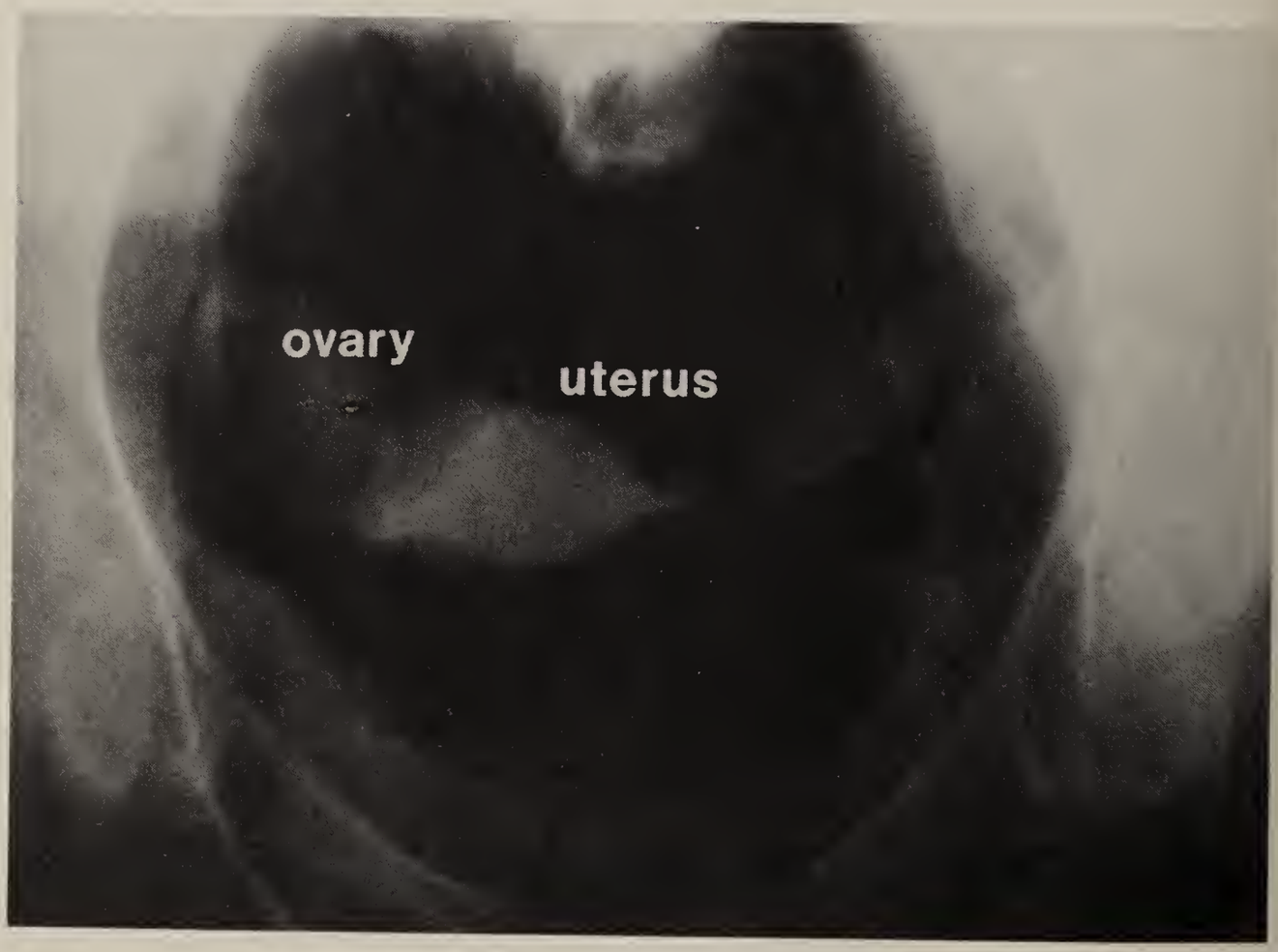

Fig. 2—Gynecography of a female pelvis. A normal ovary should appear less than one-half the size of the uterus. The overy shown is approximately the same size as the uterus, and is therefore considered enlarged.

ing it for several reasons. Laparscopy is relatively cheaper and we get a direct view of pelvic organs as well as a tissue diagnosis with ovarian biopsy.

Currently, because of the great expertise of Dr. Patel, in the Department of Radiology, we are able to obtain blood samples for hormone analysis directly from the ovarian and adrenal veins. The catheter is placed in the right femoral vein by Seldinger technique and, while monitoring it on the TV screen, Dr. Patel advances the catheter through the inferior vena cava and then into the left renal vein. Both the left ovarian and left adrenal veins flow into the left renal vein in most people, so finding their entry points in the renal vein is much simplified compared to finding the right adrenal and ovarian veins. Dr. Patel was successful on his first patient and has continued to obtain the specific blood samples we need on almost every patient he has seen with us. Thus far, in over 200 patients catheterized, there have been no complications of note (Figs 3 and 4).

Every clinically hirsute woman catheterized thus far has had elevated androgen levels either in her adrenal, ovary, or both veins. I stated previously that antecubital blood samples were elevated in only a few patients. It was most common to find an elevation of testosterone and androstendione rather than elevation of only testosterone. (Tables VI and VII). This clearly demonstrates the need to make a correct diagnosis, that is, the organ secreting the excessive androgen must be known if the correct treatment is to be prescribed. The treatment for a patient with elevated adrenal vein androgen cannot possibly be the same as that for a patient with excessive ovarian vein androgen. Thus, successful therapy which lowers androgen secretion to normal levels requires specific diagnosis and treatment.

Antecubital and inferior vena cava

Fig. 3—Adrenal venography demonstrating the catheter tip in the left adrenal vein.

Fig. 4—Ovarian venography demonstrating the catheter tip in the left ovarian vein.

TABLE VI

ANDROGEN CONCENTRATIONS IN PLASMA OBTAINED BY CATHETERIZATION OF THE OVARIAN AND ADRENAL VEINS IN 53 HIRSUTE WOMEN

Source of Plasma*	Number of Women with Androgen Excess		
	T	A	T + A
Adrenal Vein	1	5	10
Ovarian Vein	3	6	9
Adrenal and Ovarian Veins	2	9	12

*Two patients had IVC values higher than those in the adrenal or ovarian veins, suggesting peripheral conversion.

TABLE VII

PATIENT CATHETERIZATION DATA

Patient	IVC*		Adrenal		Ovary	
	T	A	T	A	T	A
E.S.	48	231	43	264	**120**	**788**
S.V.	29	280	28	272	**75**	**744**
R.M.	24	156	**348**	**7500**	28	178
J.R.	42	125	**202**	110	40	166
V.H.	44	188	**79**	**2060**	**141**	**1740**
R.R.	28	240	**190**	**1200**	**197**	**1280**
J.H.	61	347	**114**	**1279**	**121**	**1735**

Normal for women, testosterone < 54 Ng percent; antrostenedione < 280 Ng percent.

*Blood samples from the inferior vena cava and the antecubital vein will have similar androgen concentrations.

blood samples obtained close to the bifurcation are considered to have similar androgen concentrations in women. Patients E.S. and S.V. had only elevated ovarian androgen. If we had just looked at the values for androgen concentrations in the vein, we would have said these ladies had no problem; they have constitutional hirsutism. If we had treated them, as many of us who are internists tend to, with glucocorticoids first, we may not have been too successful. If we had treated them as most gynecologists treat their patients, with birth control pills, we might have provided some help temporarily. When ovarian testosterone levels exceed 74 Ng percent patients frequently complain of hirsutism.

The two patients, R.M. and J.R. have adrenal pathology. The peripheral (IVC) values and the ovarian values are normal. If we had placed these ladies on birth control pills, adrenal suppression would not have occurred. If we place these ladies on glucocorticoids we would expect to lower their adrenal androgen level.

The third group are those patients with dual problems. These ladies, V.H. and R.R. not only have adrenal but also have ovarian pathology, so they may require two kinds of treatment. If they have excessive levels of androgen in one vein and not so high in the other, one can try treating one gland and see if adequate clinical results are obtained. If hair growth continues, one may have to treat both glands. The last patient had high values even in the IVC and would represent a patient who has elevated androgens in the antecubital vein samples. With the catheterization data, it is quite easy to determine where this patient's pathology lies.

Now I'd like to turn from talking about biochemical problems to discussing a little more about the patient. We are most comfortable when we examine a patient and find something wrong. All of these patients were complaining of hirsutism and, regardless of our personal views of how hirsute they might be, the patients felt they had developed additional superfluous hair and sought treatment. Thus we

should listen to a woman when she says her hair is increasing, we do not need to await onset of shaving to work her up. This serious problem we are trying to avoid with early diagnosis and treatment.

When we examine a hirsute lady, we feel most secure if we also find a lady with big ovaries. However, I'd like to give you some data which, I hope, will dissuade you from thinking a hirsute patient is normal unless you feel big ovaries. The data in Table VIII was obtained from Dr. Archie's office records. Dr. Archie, a gynecologist, examined 45 patients, which means 90 ovaries, so he made 90 judgments on how big ovaries were. Actually he examined 88 ovaries, because one lady could only be examined under anesthesia, and those findings cannot be compared with the rest of the data. The pelvic examination results were compared with those of laparoscopic examination. On manual examination, 25 ladies had enlarged ovaries; 63 ovaries were thought to be normal in size. When he performed laparoscopic examinations, Dr. Archie found that 71 of the ovaries were large, and only 19 were normal in size. I think we can conclude that the pelvic examination, even when performed by the expert, is not conclusive (Table VIII). We must realize that many of these ladies are obese. However, the lady who weighs 100 pounds and is cooperative may not have palpably large ovaries. They may be out of reach of the examiner because they are out of the pelvis. Thus one cannot rely on examination alone, and the fact that a patient has a normal pelvic examination should not dissuade one from performing a good workup. Table IX gives some indication of which ovaries were functioning and which were not. This information comes from the pathology department. Fifteen of these ladies were ovulating, if you use the criteria listed. Twenty-six of these ladies appeared not to be ovulating, so again, one may have a hirsute lady who has big ovaries and who is ovulating each month, in spite of elevated levels of testosterone.

Unfortunately, hirsutism represents an excellent biological indication for excessive androgen production. Its presence, as reported by the patient, should always be heeded. The patient knows how much hair she had earlier and where it was located. Therefore, regardless of our thinking, we need to be advised by the patient. So again, may I point out that if the lady is complaining of hirsutism, even though she is able to have babies. please worry about her cosmetic problem and address yourself to it.

TABLE VIII

ESTIMATION OF OVARIAN SIZE BY BIMANUAL EXAMINATION AND LAPAROSCOPY

Ovaries Examined	Bimaunal Examination	Laparoscopy
Number of Ovaries Examined (45 patients)	88	90
Enlarged Ovaries	25	71
Normal Sized Ovaries	63	19

TABLE IX

SUMMARY OF OVARIAN BIOPSY RESULTS IN 41 HIRSUTE WOMEN

Physiologic Status	Number of Women	Criteria Employed
Ovulatory	15	Presence of Corpus Luteum or Albicans
Non-Ovulating	26	Absence of Corpus Luteum or Albicans Plus any two Decreased number of ova Thickened capsule Multiple cysts Fibrosis Stroma hyperplasia

ENDOSCOPY AND THE STEIN-LEVENTHAL SYNDROME

Melvin R. Cohen

My association with Dr. Irving F. Stein, Sr. goes back a long way; it began before World War II, when I was his assistant. After the war I returned and accepted his offer of a partnership with the privilege of working with a man I admired and whose interest in clinical research intrigued me.

In 1935, Stein and Leventhal[1] described the syndrome of amenorrhea associated with bilateral polycystic ovaries. The syndrome of menstrual irregularity, infertility, masculine-type hirsutism, and frequently obesity, has been well documented through the years. In 1964, Stein[2] summarized his series of 108 patients extending over a period of 34 years. He reported cyclic menses occurring in 95 percent of the patients after bilateral wedge resection. Among the 83 women complaining of infertility, 71, or 85 percent became pregnant, with a total of 181 pregnancies. In this series there were five sets of twins, which is about three times the normal rate of multiple births. Stein further stated that after wedge resection, restoration of fertility is permanent.

In my book, *Laparoscopy, Culdoscopy and Gynecography,*[3] published in 1970, I devoted two chapters in tribute to my teacher and former associate, Dr. Stein, Sr. One chapter entitled, "Gynecography," a term coined in 1943 by Dr. Stein, refers to a method of silhouetting the female organs by means of a pneumoperitoneum with or without hysterosalpingography. In this procedure, carbon dioxide is injected through the uterus as in a Rubin test, or carbon dioxide or nitrous oxide gas is injected by means of a transabdominal needle. After a liter or two of gas is introduced, the patient is placed in a modified knee-chest position and the pelvic organs are visualized, utilizing soft-tissue x-ray techniques. This simple pneumoperitoneum, when combined with a radio-opaque medium, is called complete gynecography. This diagnostic method is useful in delineating ovarian cysts, fibroids and other intrapelvic pathologic conditions. Stein relied upon this technique for the diagnosis of bilateral polycystic ovaries. The other chapter, "Endoscopy in the Diagnosis and Prognosis of the Stein-Leventhal Syndrome" is the subject of this presentation.

DIAGNOSTIC LAPAROSCOPY

In the late 1940's I became interested in culdoscopy, which I had learned from Dr. Alfred Decker of New York. With culdoscopy it was possible to visualize the ovaries associated with the Stein-Leventhal syndrome. This technique had certain limitations, especially when surgical procedures were indicated, such as biopsy. Also, photography for documentation purposes was more difficult.

We have found that laparoscopy is much more valuable than either culdoscopy or gynecography in the diagnosis and photographic documentation of the typical bilateral polycystic ovary. Such an ovary is easily visualized as enlarged, tense, globular or oyster-like in appearance and having a thickened capsule. A few blood vessels course through this thickened white capsule. Through this capsule one can see what appear to be follicle cysts ranging from 0.5 to 1.0 cm. in diameter. Frequently small cysts bulge from the surface of the ovary. There is evidence of neither recent nor old ovulation.

Melvin R. Cohen, M.D., Director, The Fertility Institute of Chicago. (Paper presented by Dr. Julian Archie, on behalf of Dr. Cohen, who was ill.)

During the past eight and a half years there were 1,425 patients from The Fertility Institute who were laparoscoped. In this group of infertile patients, about one-third had no overt pelvic disease, one-third had endometriosis, and one-third had miscellaneous findings, chiefly pelvic adhesions and the residue of P.I.D. Included in this group of miscellaneous disorders were 44 patients who had bilateral polycystic ovaries consistent with the diagnosis of Stein-Leventhal syndrome. These 44 patients represented only three percent of the total group laparoscoped for infertility.

MEDICAL THERAPY OF THE STEIN-LEVENTHAL SYNDROME

Following the report by Greenblatt et al. (1961),[4] concerning use of clomiphene citrate in the induction of ovulation, it became apparent that this was an effective drug in the treatment of Stein-Leventhal syndrome. In fact, Kistner (1965)[5] advised that it replace the surgical approach as the treatment of choice. Kistner summarized case reports of 1,704 patients treated with clomiphene citrate, 391 of whom were considered to have polycystic ovary disease. Of these 391 patients, 305 or 78 percent responded with evidence of ovulation. Roy and Associates, (1963)[6] presenting their results of the induction of ovulation with clomiphene citrate in 179 women, reported 35 patients classified as having Stein-Leventhal syndrome. Of these 35 patients, treated during 134 cycles, only six had conceptions, although 32 of them ovulated and menstruated during 117 cycles.

In a comparative study (Cohen, 1966)[7] we reported a group of 28 patients with this syndrome who were treated with clomiphene citrate during 64 cycles. Forty cycles showed typical diphasic temperature curves. We reported eight pregnancies in seven patients treated with clomiphene. Although our results with clomiphene are not as good as those reported by Stein with ovarian resection, we urge an adequate trial of therapy with this drug before resorting to bilateral wedge resection. The number of courses of therapy depends largely on whether side effects occur. The occurrence of temporary cystic swelling of the ovaries is not a contraindication to continuation of this therapy, since these cysts disappear in time. In one patient we chose to resect the ovaries immediately, rather than wait for involution, because of the size of the enlargement and because we were interested in the histopathology of ovarian changes produced by this drug.

Patients with typical polycystic ovaries seem especially sensitive to clomiphene and we urge a trial dose of no more than 50 mg. for five days or less. Prior therapy with clomiphene in no way altered results from bilateral ovarian resection.

Kistner (1965)[8] has suggested that patients who do not respond to clomiphene alone should be treated with injections of human chorionic gonadotropin (HCG). In patients receiving clomiphene therapy there is typically a spurt of mucus which occurs about seven days after the drug therapy is stopped. When this mucorrhea occurs, we perform insemination or urge coitus. The patient is examined the following day for evidence of longevity of spermatozoa. If clomiphene is apparently inducing ovulation and the patient still does not conceive, we give HCG in doses varying from 2,000 to 10,000 units, intramuscularly, at the time of the spurt of mucorrhea and continue daily until thickening of the mucus occurs.

Table I documents results of treatment, chiefly medical, of patients with polycystic ovaries diagnosed at laparoscopy. Treatment included Clomid® alone, Clomid® plus human chorionic gonadotropin (HCG), Clomid® plus menotropins (HMG or Pergonal®*) with HCG, HMG plus HCG, Clomid® with corticoids, HCG alone, and gestogens. Also included is wedge resection with pre- and post-operative medical therapy. There were 37 patients treated medically, with 11 pregnancies. Four of these returned as sec-

*menotropins (Cutter)

ondary infertility patients, with five additional conceptions. Only seven patients in this series were treated surgically. All but one had pre- and post-medical treatment with ovulatory stimulating drugs. There were three pregnancies in this small group.

Recently we have been treating those patients who have Stein-Leventhal syndrome with Pergonal® and HCG therapy, with good results. One of our Stein-Leventhal patients had her first successful pregnancy following Clomid® therapy. As a secondary infertility patient, she was treated successfully with Pergonal®-HCG and delivered quintuplets, four of whom survived.

CASE HISTORIES

Case #1—C.S. (14507). Age 22. First sen 9/1/70. She stated that she had been married three years and had been "on the pill" for 14 months. Birth control had not been used for 22 months. Since stopping the pills menses had been irregular from 33 to 42 days. Preoperative endometrial biopsy had disclosed proliferative endometrium. FSH 96 units (normal); PBI normal.

Laparoscopy, performed 10/16/70 revealed the right ovary to be approximately two times normal size, of normal shape, with a white-appearing tunic, and a few fine blood vessels coursing through it. There were a few follicles visible on the surface and there was no sign of either old or recent ovulation. The left ovary was normal size, smooth, white, and contained more superficial follicles. The impression was that we were dealing with small polycystic type ovaries. A curettage disclosed proliferative endometrium.

Clomid® was prescribed in the does of 50 mg. a day for five days and this was followed by a short, 22-day period, and she had a monophasic curve. The same dosage of Clomid,® 50 mg. a day for five days, was prescribed on day five of this cycle. On days 16 and 17 a good muchorrhea occurred with a fair postcoital test. At this time human chorionic gonadotropin 10,000 units were given, and the patient conceived during this cycle and delivered a single female child on 9/12/71.

She again returned on 2/12/73, complaining of secondary infertility. A birth control pill had been used for the first five months after delivery of her child, but she had been unable to conceive during the following year and apparantly was having irregular anovulatory cycles. She was unsuccessfully treated over a period of 14 months. During this time she had five courses of Clomid® alone, six courses of Clomid® plus HCG, one course of HCG alone, and two courses of Clomid® plus Pergonal® plus HCG. During the second course of this therapy she conceived, but aborted.

Laparoscopy, performed 9/11/73, disclosed resting type ovaries without evidence of old or recent ovulation. There were a few superficial follicles in each ovary. Pergonal® ampules two were given on nine consecutive days. This evoked a good cervical muchorrea and excellent post-coital tests. The following day HCG 10,000 units were injected. A plasma estradiol test had been ordered, but unfortunately was never performed. Forty-eight hours later the patient telephoned to complain of severe abdominal pain. She was examined, and the right ovary was found enlarged to 4x4 cm. Twelve days later she was re-examined and the right ovary had now enlarged to the size of eight cm. However, the patient was comfortable and did not require hospitalization. Three weeks after the induction of ovulation with HCG, pelvic examination disclosed a uterus the size of a two-month gestation and the probability of a multiple pregnancy. Ultrasound, performed 1/6/75, diagnosed quadruplets. She was delivered by caesarian section at eight months' gestation, on 2/9/75 of quintuplets, four of whom survived.

In summary, this is a patient in whom small polycystic ovaries of the Steinoid type were diagnosed. She initially responded to Clomid® plus HCG and had a single child. However, as a secondary infertility she failed to respond to multiple courses of Clomid® alone or Clomid® plus

HCG, but did conceive and miscarried with the combination of Clomid,® Pergonal® plus HCG. Repeat laparoscopy disclosed the absence of ovulation, and a course of Pergonal® was prescribed. With this she did develop a mild ovarian hyperstimulaiton and delivered living quintuplets at eight months.

UNSUCCESSFUL RESULTS FOLLOWING BILATERAL OVARIAN RESECTION

We have observed patients who have had unsuccessful ovarian resection for Stein-Leventhal syndrome. These patients usually have relatively regular cycles and apparently ovulate, but they remain infertile. Laparoscopy is useful in evaluating the results of such surgery, and we have found endoscopic evidence of ovarian adhesions extensive enough to prevent ovulation.

Case #2—V.K. (17040). This is an example of an unsuccessful ovarian resection. Age 30. Was first seen 3/6/74, married seven years and never pregnant. She stated that menarche was at age 13. Her menses were grossly irregular until 1970 when she had a bilateral ovarian resection for polycystic ovaries associated with Stein-Leventhal syndrome. A review of her hospital record showed that she had not responded to treatment which included thyroid, prednisone and Clomid.® At the time of her surgery the uterus was small. Both ovaries were markedly enlarged. A typical wedge resection was performed and two specimens, measuring 4x1½x1½ cm. and 2.5x1.2x0.8 cm. of tissue which had a smooth membrane and numerous follicle cysts measuring up to 0.5 in diameter were removed from the left ovary. From the right ovary there were two similar fragments of tissue measuring 3.8x1.5x1 cm. each. Microscopic diagnosis was benign fibrocystic cortico-ovarian fragments, compatible with Stein-Leventhal ovaries. In addition, an appendectomy was performed. The appendix showed focal obliterative fibrosing.

The postoperative course was febrile. However, postoperatively her periods became regular, 30 to 35 days, with dysmenorrhea, but she failed to conceive.

Laparoscopy, performed 10/22/74, disclosed marked pelvic adhesions obscuring the right adnexa and involving the left adnexa. The left tube was visualized. It showed gross kinking when indigo carmine was injected, but there was no evidence of patency. The fimbria could not be visualized. Laparotomy was immediately performed, with lysis of extensive adhesions. Both ovaries were dissected free from bowel and omental adhesions. The ovaries appeared normal. The left ovary was enlarged with a cystic corpus luteum. The fimbriated ends of both tubes were normal. The tubes were further straightened by lysing peritubal adhesions, and a small defect in the posterior leaf of the broad ligament was closed. This area was the site of a markedly adherent left ovary. At the conclusion of this procedure one gram of cortisone acetate crystals was instilled into the cul-de-sac and the abdomen closed. Postoperatively, Decadron®* and antibiotics were given, and the patient made an uneventful recovery.

Postoperatively, menses became relatively normal, occurring every 28 days; she spontaneously conceived during the month of January, 1975, and is currently pregnant.

LAPAROSCOPY AND OVULATION

Endoscopy has been of value in determining whether a patient really ovulates following resection or after therapy with clomiphene citrate. We have seen evidence of ovulation and of diffuse cystic degeneration and luteinization without ovulation following clomiphene citrate therapy.

Patients with amenorrhea, who are suspected of having the Stein-Leventhal syndrome, report to our office weekly or twice weekly for ovulation timing. We observe such patients very carefully for evidence of changes in cervical mucus correlated with the basal body temperature curve,

*Dexomethasone (Merck Sharp & Dohme)

since occasionally spontaneous ovulation without therapy may occur. Should there be clinical evidence of ovulation, endoscopy is useful in confirming the presence of a recent corpus luteum.

We recently observed a patient who had amenorrhea for six months, during which time three courses of clomiphene had been unsuccessful. Our plan was laparoscopy and probable bilateral ovarian wedge resection. At laparoscopy we found evidence of a moderately enlarged ovary on the right with a thickened capsule, typical of the Stein-Leventhal syndrome. The other ovary was globular and enlarged to almost the size of the uterus; the capsule was also thickened. This ovary was rotated with a probe and, to our surprise, a very vascular corpus luteum was on the undersurface. Laparoscopy disclosed that surgical intervention was unnecessary. This ovulation resulted in pregnancy.

UNSUCCESSFUL RESULTS FOLLOWING OVULATORY-STIMULATING DRUGS

In our current enthusiasm over Clomid® and Pergonal® let us not forget that there is still a place for ovarian wedge resection in the treatment of the Stein-Leventhal syndrome.

The following case report illustrates medical overtreatment.

Case #3—S.F. (16905) First seen 10/27/73 with the complaints of irregular menses with amenorrhea up to six months. She was 24 years of age, had been married three years and never became pregnant. Three courses of Clomid® had been prescribed for this patient before she was seen here. Further courses of Clomid® alone, Clomid® plus HCG, Clomid® plus Pergonal,®, and Pergonal® plus HCG had been prescribed over a period of nine months but no conception occurred.

Laparoscopy, performed 1/24/74, disclosed typical bilateral polycystic ovaries, compatible with Stein-Leventhal syndrome. Ovarian biopsies were performed which disclosed ovarian stroma with fibrous thickened capsule. A dilatation and curettage disclosed cystic endometrial hyperplasia. She is finally scheduled for wedge resection.

SUMMARY AND CONCLUSIONS

Laparoscopy is a valuable technique in the diagnosis of polycystic ovaries associated with the Stein-Leventhal syndrome.

During the past eight and a half years 1,425 patients at The Fertility Institute were laparoscoped; 44 patients (3 percent) had bilateral polycystic ovaries. This suggests that the Stein-Leventhal syndrome is an infrequent cause of infertility.

Medical therapy, especially Clomid® plus HCG, is an effective method of therapy for the Stein-Leventhal syndrome. It is our opinion that such medical therapy should be instituted prior to ovarian wedge resection (Table I). Further, there is no contraindication to the surgical approach after such medical treatment, provided that a period of several months elapses to allow ovarian hyperstimulation to subside.

Surgical failures do occur after ovarian wedge resection. In such patients ovulatory-stimulating drugs may be prescribed postoperatively.

Laparoscopy is a valuable diagnostic procedure after failed ovarian wedge resection. At this time it may be possible, by means of the two-puncture technique, to cut and coagulate peri-adnexal adhesions. With very extensive adnexal adhesions, repeat laparoscopy may be necessary.

TABLE I

RESULTS OF TREATMENT OF PATIENTS WITH BILATERAL POLYCYSTIC OVARIES DIAGNOSED AT LAPAROSCOPY

	Medical Treatment		B.O.W.R. Plus Medical Treatment	
(See Key Below)	Number of Patients	Pregnancies	Number of Patients	Pregnancies
C	5	1	2	2
CG	14 (1)	4 (1)	3	1
CMG	8 (1)	3 (2)	1	
MG	2 (1)	1 (1)		
CT	1	1		
G	1			
P	1			
None*	5 (1)	1 (1)	1	
TOTAL	37 (4)	11 (5)	7	3

B.O.W.R.—bilateral ovarian wedge resection.

Numbers in parenthesis refer to additional courses of treatment re: secondary infertility.

C—Clomid®

G—Human Chorionic Gonadotropin

M—Pergonal®

T—Corticoids

P—Gestogen

*Three of these were unmarried.

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VAGINAL APPROACH TO SURGICAL TREATMENT OF POLYCYSTIC OVARIES

Frederick Hofmeister

I want to express my gratitude for being invited to participate in this meeting. I have for many years been familiar with the work of Dr. Stein: When I was a general surgical assistant my mentor, Dr. Karl Friedbacher, used to demonstrate what he called "oyster ovaries" in the patients who didn't menstruate. He would wedge them and say, "That's going to help her." Well, I didn't hear much more until I came to Presbyterian Hospital and learned to know Dr. Stein and attended some of the Chicago Gynecological meetings and heard more about the Stein ovaries, which in fact were the oyster ovaries described by Dr. Friedbacher. I want to pay tribute to Dr. Stein, not only for being a good doctor and a good teacher, but an individual who recognized the opportunities to do clinical investigation, to observe and see, to record, and as a result of these recordings to come up with some fundamental basic information which has helped other people treat patients. Besides paying tribute to him as an individual, myself to him, I pay tribute to him by bringing congratulations from the American College of Obstetricians and Gynecologists for the work he has done through these many years, and hope that he will continue in good health. And, finally, coming here gives me an opportunity to say thank you to Presbyterian where, under Doctors Heaney, Allen, and Priest, I was able to learn the fundamentals of vaginal surgery which have stood me in good stead during my practice.

Frederick Hofmeister, M.D., Immediate past President, American College of Obstetricians and Gynecologists; now in practice at 10425 West North Avenue, Wauwatosa, Wisconsin 53226.

After making the decision that a Stein ovary is present, you can very easily treat this patient by the vaginal approach. I am in accord with laparoscopic examinations, with the use of Clomid,® with the use of HCG/ when indicated, and all approaches. But where surgery is necessary, I think that we have a good approach through the cul-de-sac, so often called colpotomy. Actually it is an incision into the cul-de-sac, and I have tried to stress "culdotomy" as the word to be used. We've used this not only here but we've used it also in tubal ligation. We do the wedge resection utilizing intravenous and local anesthesia.

The procedure takes about twenty to twenty-five minutes, sometimes much less than filling the abdomen with gas in preparation for a laparoscopic examination. Although I am a laparoscopist, I think there is an avenue open via the vagina too. Prior to surgery we are always careful to culture the vagina to make sure the ever-present gonococcus isn't present. This is important. Then, after our aseptic preparation, we go about the operation as you will see here. (A movie was shown.)

There are three ways to examine the pelvis through the cul-de-sac: culdocenthesis, culdoscopy, and of course culdotomy. Culdotomy is made in the loose section of the pelvis, in the most dependent part of the vagina, with an extension of the culdotomy incision if you need it. The culdotomy allows one to look and to palpate. Careful palpation gives you many of the secrets which you cannot see alone. Besides this, after the cul-de-sac is opened we use the Emmet hook (which was taught to me by Allen and Heaney) to tip and manipulate the uterus. This permits bringing the ovary into position. It can be grasped with a Babcock or a long Allis forceps. Of course meticulous closure is

important, and four stitches are used to incorporate the vaginal tissue along with the peritoneum.

The pelvis is cárefully examined pre-operatively but not only wtih the bi-manual examination—this doesn't tell you the true story of the pelvis. Recto-abdominal examination revéals many secrets of the pelvis which otherwise would not be detected. This patient is now re-prepared and re-draped, and the culdotomy performed.

The patient is put in fifteen degrees of the Trendelenburg position, the labia are sutured laterally unless there are no real redundancies. We sound the uterus to detect size and position and to verify this before doing a dilatation and curettage. We do a dilatation and curettage to determine the character of the lining of the uterus as well as the endocervix. You will see that this lining yields no endometrium. You wouldn't expect it with the Stein-Leventhal syndrome. We do a curettage first the endocervix, and then the endometrial cavity curettage. Tenacula are placed in the cervix and it is brought anteriorly. This demonstrates the position of the "vaginal hysterectomy incision." The relaxed cul-de-sac is apparent, and a very definite incision is made into the cul-de-sac. The peritoneum is spread open, and a Heaney inserted. The pelvis is palpated carefully to determine whether we have any adhesions or any abnormal pathology which we had not anticipated. The culdotomy incision is enlarged, and then a pack is placed in the cul-de-sac. This enhances the surgical exposure. The Emmet hook is placed deliberately into the posterior surface of the uterus, and the tenacula are removed. This gives mobility so that you can swing that uterus from left to right and expose the ovarian ligament. Thus the ovarian ligament is brought into view, and carefully the ovary is brought out into the vagina. And here you see an ovary which is smooth, has a thick capsule, and greatly enlarged.

The tenacula are removed, a suture was placed at the ovarian ligament and an Allis forceps was placed at the free end. A deliberate large wedge resection is made. Multi-cysts are demonstrated beneath the thick tunica in the cortex. A generous wedge is removed. One of the tricks is to remember that hemostasis is the answer. Hemostasis must be carefully achieved. We achieve it by an over-and-over suture which is parallel with the most dependent part of the wedge. If you ever had the thrill of doing an enucleation of an ovarian tumor and having what you consider is a good job and then having the patient come in for a post-operative examination with an ovary twice as big because she has sustained a hematoma, you know that the name of the game is controlling hemorrhage. After you have this suture placed, you can see that the hemorrhage from the ovary is controlled.

Now then we go through the edge of the incision and use a Heaney stitch to assure that this suture will not slip, and then go back and lockstitch to approximate the defect. There is no bleeding, and the wedge resection has been completed. The ovary is returned to the pelvis and we show the opposite ovary and the resection only to demonstrate that instead of holding it with an Allis forceps we can insert two sutures and accomplish visualization that way. When I put a suture in like that, I don't remove the suture, I just don't pull it out; I tie it, because by pulling it out, we would have the opportunity again of causing a vessel to bleed. This again demonstrates the thick tunica, the multiple cysts—you can see them very clearly as we go along, and then again the double layer closure will be done.

Rush-Presbyterian-St. Luke's has a tremendous heritage in its great renown for vaginal surgery. It's been recognized throughout the world and a debt is owed to Heaney, Allen, Priest, Kantor, Klawans, Baum, Draa, and Boysen. Hopefully this will be perpetuated.

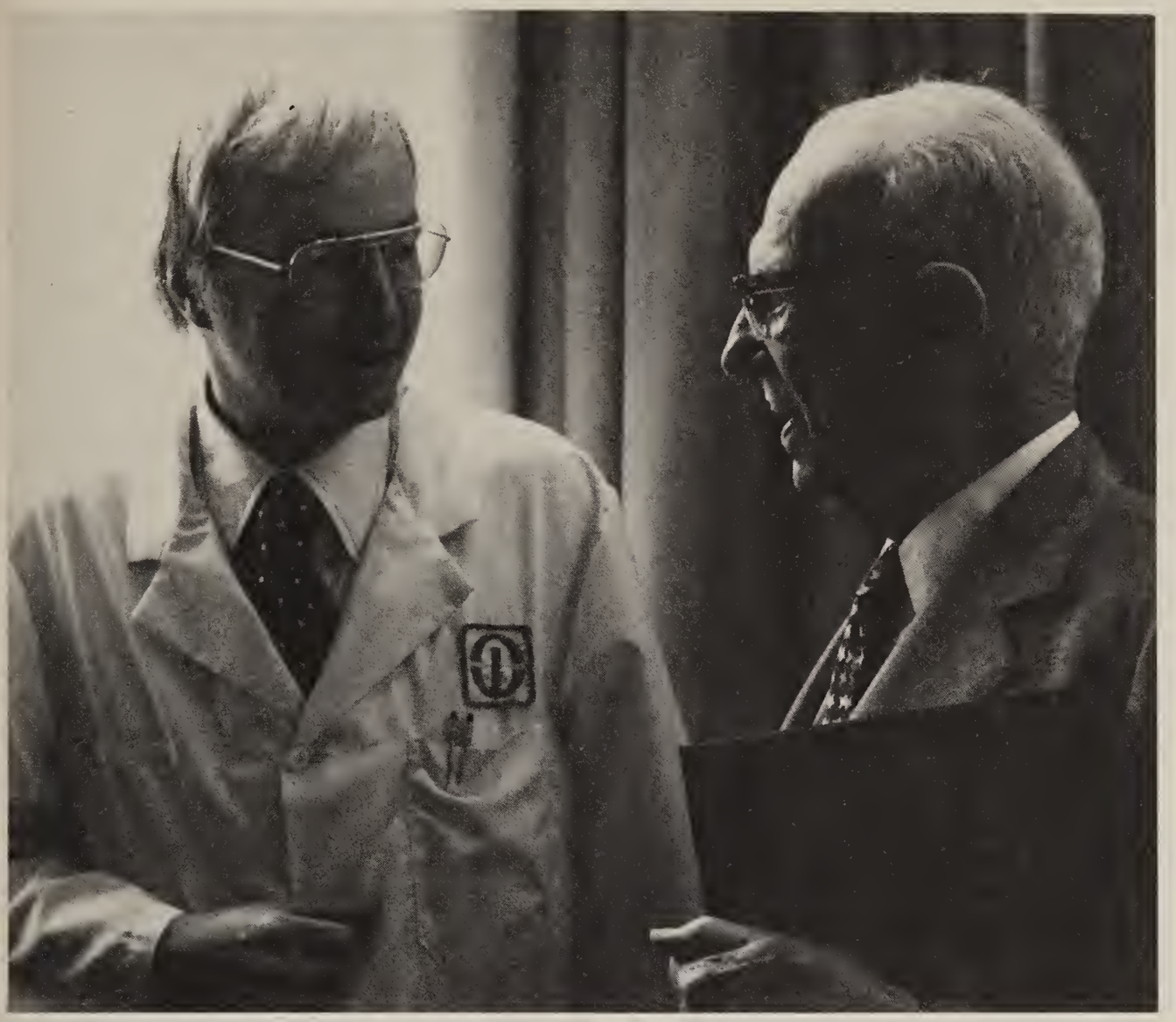
We reapply the tenacula. We close the culdotomy incision; it is important to go through the mucous membrane, pick up the peritoneum, then go into the corner and be sure that the defect in the corner is picked up, so that you don't have a

bleeding spot there. Then go through the peritoneum and the posterior wall and, yes, go through all the layers.

What do I use to close the culdotomy incision? I use either 2.0 dexon, which has proved very good for me, or I use #1 chromic catgut. The patients' recovery is rapid and they leave the hospital the next morning.

What about prophylactic antibiotics? Yes, we start this the day prior to the surgery and continue through the hospital stay.

The case I have shown is a 21-year-old multipara with amenorrhea and infertility. A perineotomy was necessary prior to the culdotomy. This operation was done in 1966. She became pregnant and delivered in 1970. In 1973 she had a miscarriage. She is due to be delivered of her second baby in May 1975. She has requested tubal ligation, and so we've gone the whole cycle, amenorrhea, Stein ovaries, pregnancies, and now tubal interruption.



Dr. Stein (right) with George D. Wilbanks, M.D.,

PANEL DISCUSSION

Dr. Wilbanks: All the speakers have consented to be on the panel. In Dr. Cohen's absence we have drafted Dr. Anthony Scommegna, Chairman, Department of Obstetrics and Gynecology at the Michael Reese Medical Center. To open the panel I will call on Dr. Scommegna for his comments.

Dr. Scommegna: Thank you very much. My congratulations to the participants in the symposium. The Stein-Leventhal ovaries as part of the hirsute syndrome are a thorn in the flesh of the endocrinologist, because our knowledge is meager on this subject. We still do not know the etiology of this syndrome. Perhaps some of the panelists may address themselves to this. Although we have medical treatment for this disease, such as clomiphene citrate, Perganol®* and human chorionic gonadotropin, the results with medical management are not always as good as with wedge resection. So while medical treatment may be used, certainly we will not discard wedge resection of the ovaries.

Dr. Northrop, you have gone to great length to make a diagnosis of the ovarian versus the adrenal origin of hirsutism. By doing so you identify a methodology of treatment by adrenal or ovarian suppression. Have you re-catheterized patients while treatment is in progress? Do you

*menotropins (Serona)

treat patients, in whom the ovarian vein was the origin of increased androgen, with corticosteroids, and the patients with an adrenal origin of excessive androgen with sex steroids, estrogen and/or progesterone? Kirstner, using the same technique as in your series, found in one third of his patients that excessive androgen was of ovarian origin. However, administration of dexamethasone did cause a decrease in the androgen production in some. I question whether you can really choose the specific treatment to apply in each patient. I would also like to ask whether you ever had the opportunity of using or measuring dehydroepiandrosterone, which in some cases of adrenal hirsutism may be elevated, either as the free form or sulphate conjugate? Perhaps you may wish to say a few words about sex-steroid-binding proteins.

Dr. Northrop: Let me address myself to the question which was stimulated by Kirstner's recent paper which indicated that one-third of hirsute patients will show ovarian stimulation by adrenocorticotrophic hormones (ACTH). Prior unpublished studies by us, using synthetic Cortrosyn, showed increase in testosterone concentration of ovarian blood in one fifth of the patients. There is considerable crossover in stimulation of ovaries and adrenals. We can only say now that if the ovarian vein is high in androgens, we should treat the patient as if the ovary is primarily at fault. We treat the ovary specifically by supression (estrogens) or wedge resection. If the levels are high in the adrenal vein, we usually treat the patient with glucocorticoids. I have recatheterized some patients who did not respond to glucocorticoid. Sometimes a dose that will be suppressive while the patient is in the hospital, calm and supported by the medical personnel, will not be suppressive outside of the hospital where the patient may be under some stress. In other words, additional stress may force us to use more than normal suppressive glucocorticoid doses.

Also, there is the patient who only weighs 90 or 100 pounds, with excessive adrenal androgen, who may become Cushingoid on a daily dose of 5 mg of prednisone. The usual amount we utilize for adrenal suppression is 5 mg prednisone at night and 2.5 mg in the morning. The night dose is the larger in order to stop the morning ACTH surge. We try to use the lowest dose possible. I have had patients get Cushiongoid on this dose and have been forced to lower it. I have also had three patients become psychotic even on these low doses, and we have not been able to continue treating them with this drug.

If the patient has only excessive ovarian androgen, and there is no adrenal component, I advise wedge resection. This is curative. We have catheterized patients after wedge resection and found that testosterone values are normal. If you place patients on birth control pills, which in a sense "puts the ovary to sleep," you usually do not obtain suppression, and surgery must be resorted to later. If the patient has both adrenal and ovarian excess androgen, I suggest medical suppression first with glucocorticoids. If this is not effective, wedge resection is recommended. I remain concerned about patients on long-term therapy with steroids.

The other question is the matter of testosterone binding. The amount of estrogen present is going to determine the amount of testosterone binding. You are all familiar with this in terms of the elevated PBI, when a patient is pregnant. There are more binding proteins available when estrogen levels are elevated, and therefore if you were to measure copper or iron or cortisol in the presence of estrogen you will find that all of these transporting proteins are increased. If you give the patient birth control pills or estrogens you will find that the testosterone-binding protein is increased. Since the physiologically active form of testosterone is not lowered, we are really interested in the amount of free testosterone. Some patients not only have high levels of testosterone but also have a high level of estrogen. There are patients who make a lot of precursor, and

there is no inhibition in conversion of testosterone to estrogen. These patients have higher plasma levels of total testosterone, but not necessarily more free testosterone. Only one percent of the total testosterone measured is unbound or free or in the active form. At the present time we cannot obtain free testosterone levels either at this institution or commercially. Thus we have to look at the clinical symptoms and the biochemical levels and make the best judgment we can in directing patient care.

Dr. Wilbanks: Dr. Hofmeister, in view of the results shown by Dr. Cohen and by others, of the possible adhesions of the ovary to the pelvic wall and tube, do you have any laparoscopic follow-up patients who have had ovarian wedge resections? Also would you advocate ovarian wedge resection by "culdotomy" in the typical 250-pound nulligravida?

Dr. Hofmeister: To your second question, the answer is yes. A vaginal approach is best for the very obese patient. The nulliparous patient does not present a problem. Surgical exposure is aided by performing a "culdotomy" or a perineotomy.

Dr. Scommegna: I wondered if the perineotomy was done prior to the cul-de-sac incisions?

Dr. Hofmeister: Yes. In showing the movie, I just wanted to surprise you with the repair at the end. I did not want to tell you why it seemed so easy. An important point in all vaginal surgery is to place the patient in the proper position. Another secret is the use of a 15-degree Trendelenburg position.

Now what about the adhesions? When do I laparoscope a patient? When do I further investigate her? I do not do anything to her if she is menstruating normally. If infertile, and she is menstruating normally, if the sperm are normal, and that of course is 45 percent of the problem in infertility, and she still does not get pregnant, then I think we ought to investigate by laparoscopy. If the patient has been on Clomid® before she is wedged and then does not menstruate, putting her on Clomid® after wedge resection very frequently initiates menstruation.

I always insufflate the tubes prior to any other procedure, utilizing iodized oil. I have not had the problems of adhesions, and I think they will become less if we use appropriate antibiotic therapy.

Dr. Wilbanks: Any questions from the panel? Any rebuttal?

Dr. Scommegna: First of all, as to the adhesions post-operatively, in addition to Dr. Cohen's use of cortisone I add phenergan, 25 mg, two every six hours for about six days.

I am much more interested in the comment about the hirsutism with wedge resection of the ovaries, or did I misunderstand you? You said the testosterone level dropped after the wedge resection, and that this was your way of handling the hirsutism when the cause was excessive androgen in the ovarian vein. Reports in the literature agree that the testosterone level may drop, yet the metabolic defect is still present. Studies on patients after wedge resection showed the same metabolic defect even though the patient was ovulating. At laparotomy or laparoscopy, 25 percent of the patients with Stein-Leventhal syndrome will have a corpus luteum and they do not ovulate regularly. The half-life of testosterone which is increased in the hair follicle of these patients is unaltered by wedge resection of the ovary.

Dr. Northrop: This is why you cannot select which patient to wedge on any other basis than to operate on the patient in which elevated testosterone is known to be coming out of the ovarian vein; in other words, there are no dynamic tests or pooled samples that will properly select

patients who have elevated ovarian levels of androgen. If the amount of testosterone to which we are exposed is reduced, then we will not be stimulated as much as when the total daily secretion was higher before surgery. Obviously, if excessive androgen is being produced in the adrenal gland and the ovary is wedged, poor results would be expected. Thus patient selection is important.

Dr. Wilbanks: Who would you say the right people are?

Dr. Northrop: I would say the right people are the ones who have high levels of testosterone in the ovarian vein.

Dr. Scommegna: Our experience in measuring peripheral testosterone is that after wedge resection, testosterone goes down and it stays down for about three months; then, even though the patient is still ovulating, maybe menstruating, the testosterone goes up. I think this is not unique in our experience; it has been widely reported. I think the catheterization study is an excellent way of approaching the problem from an investigational point of view. I do not feel that it adds very much to the practical way of handling patients. I say that because administration of dexamethasone in patients who have been demonstrated to have a selective testosterone increase in the ovarian veins showed a drop in the testosterone when catheterized again. There are also problems with catheterization. It is easy to catheterize the left side and difficult to catheterize the right side. We assume that if there is hyperplasia on one side there will be on both sides. There are problems in placement of the catheter. One might get a mixture of blood that is not pure gonadal or adrenal in origin. There are also problems of stress. Thus one may show an accentuation in the adrenal production and mimic an adrenal problem. I was interested to know that you dropped the pneumogynocography because it had cost $350.00. I would like to know how much this workup costs?

Dr. Northrop: Well, the entire study costs considerably more than that, but it tells you ten times as much. Many times one is diagnosing a patient who has a mixed problem (ovarian or adrenal pathology). I do not think this can be discerned by any kind of dynamic testing. The patient who has both adrenal and ovarian vein androgens might be the patient you are talking about, who has decreased androgen for about three months and then, a short time later, has elevated levels again. I think there is no peripheral type of sampling that you can perform to diagnose the source of androgen. I would suggest that physicians not use dexamethasone because this a short-acting glucocorticoid compared to prednisone.

Dr. Scommegna: I want to ask you one more question about the hirsutism. How would you evaluate the decrease in the hirsutism after the wedge resection?

Dr. Northrop: Once a hair fiber is stimulated to do its thing, it is going to do it for a long time. I have quite a few male transexual patients in whom it has taken about a year and a half of good levels of estrogen in order to get some reduction in the rate of hair growth. I tell a patient when I see her that therapy will probably not reduce her hair if she has been shaving more than one year. The patients who have been hirsute or who have had terminal hair fibers for less than a year frequently get excellent remissions. A patient who has been shaving for more than a year will probably not get a total remission. I point this out very clearly to patients and their parents. I clearly state that what we are going to do is put them in a physiological state so that electrolysis will be effective. Over a period of time they may see some thinning of hair in the area where electrolysis is not applied. I also inform them that if they have the electrolysis only, without stopping the source of the androgen, it will not be successful. We do not tell patients that they are going to be cured by what we are doing. We tell them that they will not get

worse, and that we can help them reduce their current quantity of hair. I am very forceful about this on the first visit because I do not want anybody saying, "Look, you have done all this to me and you have not cured me." I repeat it two or three times so that there will be no doubt in their minds that all their hair is not going to drop out when we get through, unless they are fortunate.

Dr. Scommegna: What criterion do you use to demonstrate that the hair growth has decreased? Do you have a number of patients who have been through electrolysis a lesser number of times after surgery than before? I mean, how are you measuring this decrease in hair growth?

Dr. Northrop: Many patients do not have the problem at all any more after they have done all the things suggested. We have only been studying this type of patient for two-and-a-half years, so we have to go about seventeen years before we can write a twenty-year followup.

From audience: Is there any advantage in keeping the wedge wound open rather than closing it?

Dr. Hofmeister: This has been done, but not by me. I think if you are going to avoid adhesions, you have to have a meticulous closure and meticulous hemostasis. I would like to ask, have you used *Dexon?* You advise a non-absorbable suture. Well, Dexon is a very long-lasting suture, and, for me, I would rather see that used on the ovaries than a non-absorbable suture.

From audience: I have used Dexon. I do not like the way it feels when I tie knots.

Dr. Alex Tulsky: I would like to divert the discussion, if I may, to the pathology of the polycystic ovary, which we have not touched on at all. You know, when Stein and Leventhal first discussed this problem, some forty years ago, they described the pathology very specifically. This afternoon we have seen typical pictures of classical Stein-Leventhal disease. There are a number of enlarged ovaries which are frequently mistaken for Stein-Leventhals and this confuses long-term statistical results. As an example: Dr. Kistner, if I heard Dr. Archie correctly, reports 391 cases of polycystic ovaries. Dr. M. Cohen reports three percent of 1,400 laparoscopies, which is much more characteristic, in my opinion, of Stein-Leventhal. I myself have laparoscoped perhaps 100 women with hypomenorrhea and infertility, and I did not see more than one or two percent of so-called Stein-Leventhal. The others are either inactive ovaries or what I choose to refer to as multicystic ovaries. Multicystic ovaries may be enlarged but there certainly is no thickened tunic, and when biopsies are done on these gonads, you find some small follicles. Trying to wedge these, of course, would be inappropriate. Hopefully the use of Clomid® or one of its variants can stimulate these patients to ovulate. We have to realize that not every large ovary, not every white ovary, is the type we have been talking about all afternoon.

Dr. Hofmeister: There are multiple variations of large ovaries. The criteria which we established are the presence of multiple cysts, the thickened tunica, and corticofibrosis. Another example that confuses the diagnosis is illustrated by the following patient: In a woman 23 years old I did an endometrial biopsy after I found enlarged ovaries. I found a carcinoma within the uterine cavity, and she still was not bleeding. I performed a wedge resection. I did not remove her uterus. On followup she has reinstituted normal periods. The lesion has reverted to normal endometrium. This is the kind that Hertig and Kistner described. Finally, a very obese girl, which you alluded to before, had a history of menorrhagia. I did my preliminary evaluations and wedged both ovaries for biopsy purposes. I found they had the typical appearance of the Stein-

Leventhal ovary. She reverted to normal periods. You can have hypermenorrhea in some few patients. You can have amenorrhea, you can actually have a malignant or premalignant state.

Dr. Wilbanks: I think perhaps the term that Don Christian used, the "mystic cystic" syndrome, might apply here. Dr. Archie, will you comment about the pathology?

Dr. Archie: As far as Dr. Cohen's paper is concerned, I am assuming that the diagnoses were made on ovarian biopsies, as I think were Dr. Kistner's, and so were ours. We have seen an ovary which appears to be a polycystic ovary consistent with Stein-Leventhal, and subsequently have had negative pathology reports. There is a problem in laparoscopy, because on biopsy one does not get a large amount of tissue. Our pathology department has been quite definitive in its diagnosis of Stein-Leventhal ovaries.

Dr. Scommegna: I do not see the difficulty in pinning a diagnostic label. It means very little to this particular syndrome. We might classify it and say, yes, it is a Stein-Leventhal ovary, or no, it is not a Stein-Leventhal ovary because it conforms or does not conform to what Stein and Leventhal described 40 years back. We know very little now about what the mechanism or the pathogenesis is of this syndrome. I think it is much more important, through the kind of studies that have been presented here, to identify the endocrine profile of these patients. Where is the problem—is it the adrenal, is it the ovary? It is best, at least, to attempt to settle that.

I suggest a symptomatological approach. If the patient is not complaining of infertility, we would want to give her an estrogen-progesterone combination, pill, if she has hirsutism, but this is not going to correct infertility. If she is 16 years old and hirsute, certainly we are not concerned with stimulating ovulation in this patient—we are more concerned with the hirsutism. Not knowing the etiology of the syndrome, I think we should use the symptomatologic approach and treat whatever symptomatology we can.

From audience: I would like to ask the panel about how to treat the Stein-Leventhal syndrome in a married woman who wishes to get pregnant, and what can be done for the young woman, unmarried, who does not want to get pregnant and does have the disease? What would be the best approach to these patients?

Dr. Hofmeister: Well, I just treated a young woman with the Stein-Leventhal syndrome and amenorrhea. She was practically "jumping out of her skin" with concern. Her periods were re-established, she got married, and I have just performed tubal ligation because husband and wife have mutually agreed that they do not want to ever have babies.

Dr. Northrop: I have three patients from the surrounding communities, who are 39, 40, and 37 years of age. These ladies, I verified from the local gynecologist, had ovarian wedge resections some 20 years ago in order to correct classical Stein-Leventhal ovaries. The women have remained in good health and have managed to have three or four babies in each family. They have now returned at these advanced ages, saying, "I have the same problem back; I am getting hair again." Examination confirmed that the hair problem had returned. The ovary was shown to be the source of androgen in these ladies, so I put them on birth control pills. However, they developed diabetes and hypertension, and I therefore returned the patients to their gynecologists for ovarectomy, as each patient had completed her family. Thus, with wedge resection, some 20 years of reproductive time was bought for these patients.

From audience: What is the reported incidence of return of the symptomatology after wedge resection and established ovulation?

Dr. Northrop: I think that Dr. Stein has said that it does not happen, but I have the three patients I just told you about.

Dr. Archie: I do not have any figures on recurrence of symptoms, but it definitely does occur. I cannot give you any time interval. I think with the wedge resection you are buying time.

From audience: Why do you limit the Clomid® to 50 mg? I think you said 50 mg for five days and that you did not go to any higher dosage. Why did you limit it?

Dr. Archie: Dr. Cohen feels patients with polycystic ovaries are very sensitive to Clomid.® He has used very high dosages, but he has found that by reducing it he gets a better response.

From audience: If he does not get a response at 50, does he increase the dose (Clomid®)?

Dr. Scommegna: He does. You see, the problem is that the patients who do well on wedge resections do not come back, and the ones that do not do well do come back. We see the patients in which the wedge resection has failed. Perhaps our view is a little bit more guarded than that of Dr. Stein's. He said that all his patients did not have recurring problems. Our view is a bit more biased. We see a very significant percentage of patients who do have amenorrhea and recurrence of symptoms, following wedge resection. Meanwhile, hopefully, you can "sneak" three or four pregnancies for these girls, and the problem of fertility is solved.

Dr. Northrop: Quite often you can trick those kinds of patients into cycling with Clomid.®

Dr. Scommegna: Right.

From audience: Occasionally we have a patient who had a Stein-Leventhal ovary and had it wedge resected, and she wants to become pregnant. After a while she gets pregnant, the syndrome recurs, she subsequently wants to get pregnant again. How many times can her ovaries be wedged?

Dr. Hofmeister: I do not think that is a frequent situation. I have not seen a great number of women who have had a re-establishment of normalcy, have had the babies, and then have a Stein-Leventhal ovary occur for a second time.

Dr. Wilbanks: Let us close with a practical what-do-you-do. Dr. Scommegna, would you start?

Dr. Scommegna: Some of these patients may have a polycystic ovary syndrome and may not have hirsutism, they have amenorrhea or normal menorrhea wtih menorrhagic episodes or menorrhagia interspersed, without hirsutism. From the practical point of view, they are not ovulating. They may have increased androgen, but because of the decrease in end-organ sensitivity, they do not have hirsutism. The hirsutism is an end-organ manifestiation. So you may have a moderately or mildly increased androgen, especially at the onset of the disease without hirsutism. In these cases I have used just the cyclic progesterone, once every five weeks. I use Provera®* 10 mg per day for seven days. In patients who have hirsutism along with the unopposed estrogen or oligomenorrhea, I have used estrogen-progestorone combination. I use mildly estrogenic types, Enovid,® Enovid-E,® or Enovid® 5 mg. I continue this regime until they are ready to fall in love, get married, get pregnant, and have a baby, not necessarily in that order.

The wedge resection has complications, at least in our hands. Again, we may be biased because we see the problems. We have mainly a referral practice. We see the problems that Dr. Cohen sees as well, which are adhesions and recurrence of the syndrome. The syndrome does recur, and this is the reason we do wait on the wedge resection until fertility is desired and after the failure of clomiphene treatment.

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*Medroxyprogesterone acetate (Upjohn)

Dr. Wilbanks: Dr. Archie or Dr. Northrop, would you summarize how you would treat these patients of Dr. Sheikh's? We have two patients, one who essentially has the amenorrhea and is not hirsute, and another who is hirsute.

Dr. Archie: If she is hirsute, we wedge those patients who show large amounts of testosterone in their ovarian veins. We see an extremely biased population. We get an extremely large number of hirsute patients. You are not solving their problems by giving them birth control pills.

Dr. Northrop: Treatment depends on why the patient comes to see you. Our patients almost always have come to us specifically because, "I did not have hair before, and I do now, and what can I do about this?" I do not think one can wait to treat this patient until she decides she wants to get pregnant, because she may never get the opportunity to get pregnant. She may never get near a gentleman because of this big beard! I think you have to treat first things first, and I do not see why a woman should continue to have body hair and facial hair when it is a problem we may be able to help her eliminate. So if we have a patient who consults us because of hirsutism, we determine the source of the excessive androgen and we wedge her if it is coming from the ovary. If the patient is seen because of infertility or irregular periods, her workup is an entirely different thing, and treatment is designed to correct her abnormalities. I think we would agree that we probably would not wedge such a patient until she wanted to have a baby.

Dr. Wilbanks: Where is the place for Clomid® in the treatment of these patients?

Dr. Northrop: I am not sure what the place of Clomid® is in the treatment of Stein-Leventhal syndrome because we have not found elevated gonadotropins as reported in the literature. If you have a patient that already has high gonadotropins, why are you trying to get it higher with Clomid®? You might say, we will make a spike and get the patient to start cycling, but my experience with Clomid® is that they do not always do this. I certainly think the use of Clomid® is very good in the patient who has been treated and who does not cycle. You frequently can trick her ovaries into ovulating with this drug. I do not think that it will take the place of ovarian wedge resection because it will not lower high levels of androgen to normal.

Dr. Scommegna: I am rather interested in your approach to the problem because in an unselected series, and I say unselected in the sense that we did not know whether androgen was from the ovary or from the adrenal, wedge resection was effective, as far as improving hirsutism is concerned, in up to 15 percent of the patients. We all would be interested in the long-term followup of your patients. Can you predict the patient who will improve by wedge resection by demonstrating the ovarian origin? As far as chlomiphine is concerned, we use chlomiphine prior to wedge resection, having reserved resection for the patient who has failed on clomiphene. The mechanism, of course, is by stimulating the hypothalamic-pituitary axis and getting an FSH rise.

Dr. Wilbanks: Dr. Hofmeister, as our senior member of the panel, would you like the last word?

Dr. Hofmeister: HELP!

Dr. Wilbanks: Thanks to the panelists, and thank you all for coming.

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ON THE PATHOPHYSIOLOGY OF SEVERAL DOPAMINE RELATED DISORDERS

David I. Margolin
Harold L. Klawans

ABSTRACT. Amphetamine-induced psychosis and movement disorders, as well as levodopa-induced movement disorders are linked to the increased activity of dopamine in the brain. Recent animal work suggests that these disorders may reflect chronic agonist-induced hypersensitivity to dopaminergic stimulation. In contrast, the hyperkinetic syndrome manifests a disorder of attention which may be related to decreased activity of dopamine in the brain.

INTRODUCTION

Diverse disorders such as Huntington's chorea, Parkinson's disease, levodopa-induced dyskinesias, tardive dyskinesias,[1] amphetamine-induced psychoses and movement disorders[2] and Guy de la Tourette's disease[3] have been linked to the altered activity of dopamine at dopamine receptor sites within the brain.

This paper discusses recent advances in the study of several disorders associated with increased activity of dopamine in the brain, namely levodopa-induced dyskinesias and amphetamine-induced psychoses and movement disorders. In addition, evidence is presented implicating dopaminergic hypoactivity in the pathophysiology of the hyperkinetic syndrome.

This research was supported by grants from the United Parkinson Foundation, the Michael Reese Research Institute, and the Boothroyd Foundation, Chicago, Illinois. The first author was supported by a grant from the Northside Ladies' Auxiliary of the United Parkinson Foundation.

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LEVODOPA-INDUCED DISORDERS

Levodopa-induced dyskinesias represent the most common dose limiting side effect of levodopa therapy for parkinsonism. Up to 80 percent of parkinsonian patients experience abnormal movements within 18 months after beginning levodopa therapy.[4] Most commonly these dyskinesias are cephalic in location producing lingual-facial-buccal dyskinesias and involuntary movements of the head. Less commonly the dyskinesias involve truncal and axial musculature.

The pathology of parkinsonism involves degeneration of dopamine containing cells in the substantia nigra[5,6] and subsequent depletion of dopamine in the corpus striatum.[7] This has led to the suggestion that dyskinesias associated with the use of levodopa in parkinsonian patients reflect a denervation hypersenitivity[1,4] of striatal dopamine receptor sites.

The concept of denervation hypersensitivity explains the fact that most parkinsonian patients receiving levodopa therapy frequently develop dyskinesias while normal persons receiving equivalent amounts of levodopa rarely do.[8] However, several characteristics of levodopa-induced dyskinesias are not accounted for by this theory.

(1) A linear relationship has been observed between the duration of levodopa therapy and the number of parkinsonian patients who develop dyskinesias.[1,4] Based

upon the denervation hypersensitivity hypothesis of levodopa-induced dyskinesias, one might predict that as the dopamine levels increased in these patients there would be a reversal of the hypersensitivity and a leveling off or a decrease in the incidence of dyskinesias. It has been found, however, that the incidence of dyskinesias continues to rise as long as two years after the initiation of levodopa therapy.[1] Even if the denervation hypersensitivity were long lasting one would not expect to see a continued rise in the incidence of dyskinesias after a steady state level of dopamine had been reached.

(2) Once levodopa-induced dyskinesias begin they often increase in severity with continuation of the same dose of levodopa. The movements may also spread so as to include additional parts of the body.[9]

(3) In many cases where the prescribed amounts of levodopa have been reduced because of the severity of dyskinesias, the movements have recurred even at lower levels.[10]

(4) In many patients there tends to be a progressively decreasing time interval between the ingestion of levodopa and the appearance of dyskinesias. When the patient first experiences dyskinesias they tend to occur one to two hours following the ingestion of levodopa. Later in the course of treatment the dyskinesias may occur within minutes after taking the drug.[9]

Recent work which has been conducted in our laboratories offers some insight into a possible explanation for these puzzling observations. These investigations involved the effects of chronic dopaminergic stimulation on the production of amphetamine-induced sterotyped behavior in guinea pigs.

Amphetamine-induced stereotyped behavior, a species-specific phenomenon occurring in most mammals has proven to be a useful model for the studying of a number of human movement disorders including Huntington's chorea, tardive dyskinesias and Parkinson's disease.[11] The model of amphetamine-induced stereotyped behavior is appropriate for the study of levodopa-induced dyskinesias since both phenomena are believed to be a reflection of increased dopamine activity within the corpus striatum.[1,12] Chronic dopaminergic stimulation is important, since levodopa dyskinesias develop after prolonged use of the drug.

When housed in metal cages guinea pigs display stereotyped behavior manifested primarily by continual chewing of the bars of the cage in response to both amphetamine and apomorphine stimulation. In this series of experiments, two groups of young male guinea pigs were given daily subcutaneous injections of 4 mg/kg d-amphetamine sulfate (Group A) or 5 mg/kg d-amphetamine sulfate (Group B) for three weeks. Immediately following the injections the animals were observed for the appearance of amphetamine-induced stereotyped behavior over a 60-minute period. During this time the intensity of stereotyped behavior was rated on a scale of 0 to 4+ similar to that suggested by Ernst.[13]

Animals in both groups displayed a decreasing latency and increasing intensity of stereotyped behavior with repeated d-amphetamine administration. For example, only one of 12 animals in Group A displayed a high degree ($>$ 3+) of stereotyped behavior with the first amphetamine injection. By the fifth day of amphetamine administration nine of the 12 animals developed 3+ stereotyped behavior. Furthermore, while 40 minutes were required for the nine animals to reach 3+ stereotypy on day 5, it required only 20 minutes by the eighth day of amphetamine administration (Fig. 1). As seen in Fig. 1, the overall degree of stereotyped behavior developed over the 60-minute period increased each day. As shown in Fig. 2, Group B animals displayed a sharper decrease in latency and a greater intensity of chewing.

At designated times, ranging from 3 to 34 days following the end of the three week pretreatment with amphetamine, animals in both groups were treated with 4 mg/kg d-amphetamine or 0.2 mg/kg apomorphine. Such dosages usually do not

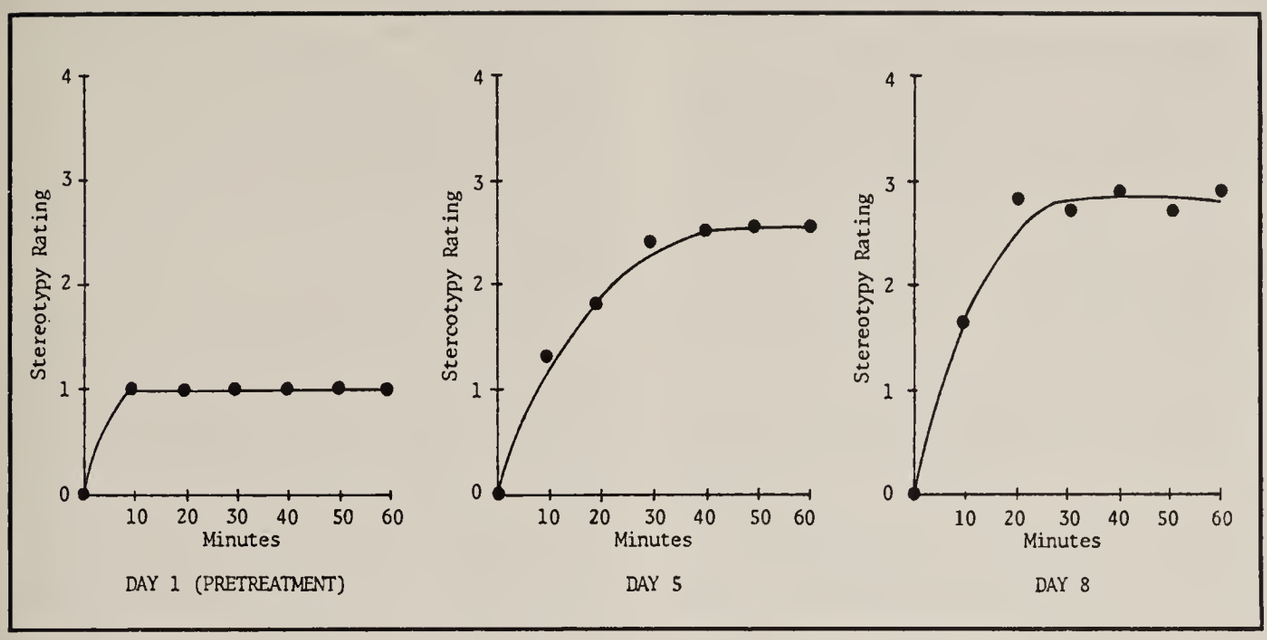

Fig. 1—Time course of amphetamine-induced stereotyped behavior in Group A animals.

produce a high degree of stereotyped behavior in untreated guinea pigs and are thus considered to be subthreshold.

In both Groups A and B animals, however, the 0.2 mg/kg subthreshold dose of apomorphine produced $\geq 3+$ stereotypy at 3 and 10 days following the end of the pretreatment period, and a subthreshold dose of 4 mg/kg amphetamine produced $\geq 3+$ stereotypy at 7 days following the pretreatment period. Animals in Group B, tested 34 days after their last dose of d-amphetamine, developed $\geq 3+$ stereotyped behavior in response to subthreshold apomorphine administration. In all cases the results were statistically significantly different from untreated controls. A more detailed description of this series of experiments is reported elsewhere.[9,14,15]

These data suggest that the chronic administration of d-amphetamine produces a long-lasting, dose related hypersensitivity to subsequent dopaminergic (via amphetamine or apomorphine) stimulation. This hypersensitivity is manifested

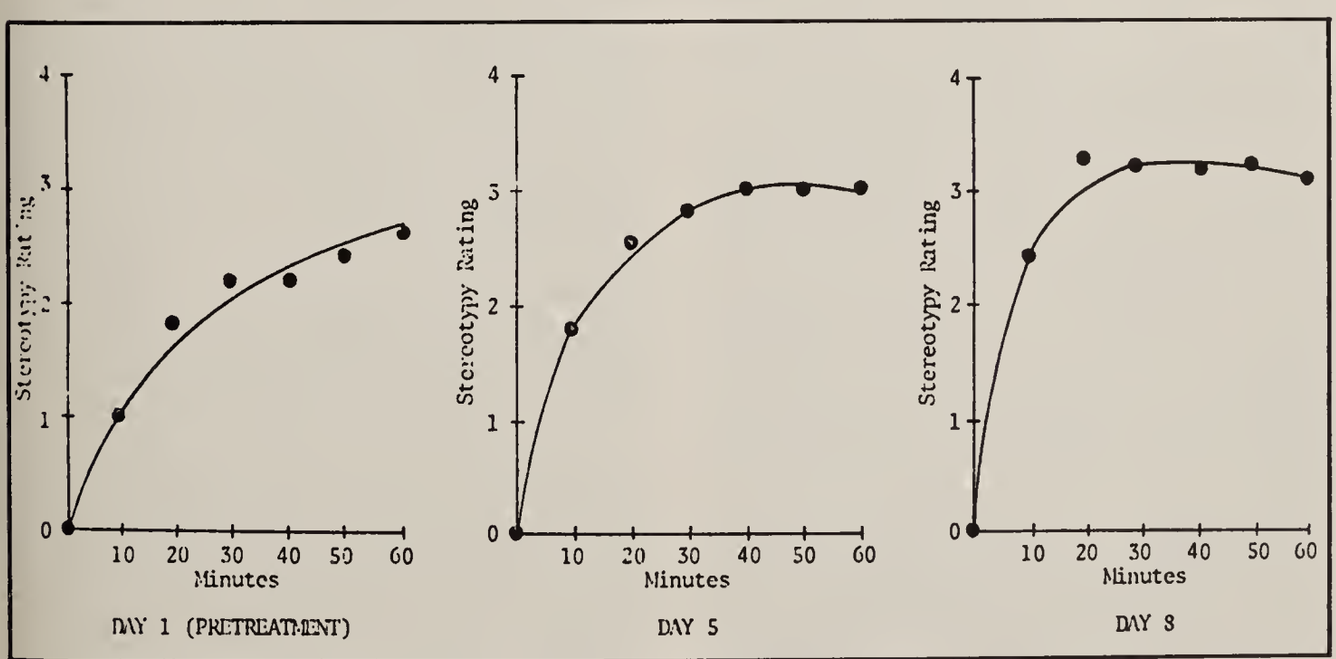

Fig. 2—Time course of amphetamine-induced streotyped behavior in Group B animals.

by a decreased latency, increased intensity, and decreased threshold for the production of stereotyped behavior.

Amphetamine is an indirect dopamine agonist which acts by increasing the concentration of catecholamines at the receptor site.[16] Apomorphine, on the other hand, is believed to exert agonism directly at the dopamine receptor site.[13] Therefore, the fact that guinea pigs were found to have an altered response to apomorphine as well as to amphetamine following chronic amphetamine pretreatment suggests that an alteration of the postsynaptic receptor site had taken place.

It is unlikely that the above results are due to an accumulation of dopamine during the pretreatment period, since it has been shown that chronic d-amphetamine administration produces decreased levels of brain dopamine and norepinephrine in rats[17] and guinea pigs.[18] It is also unlikely that an accumulation of amphetamine was responsible for the increased sensitivity, since the half-life of d-amphetamine in guinea pig brain and plasma is 2.5 to 3.1 hours[18] and the hypersensitivity was found to last for at least 34 days in this experiment. It may be argued that an unusual amphetamine metabolite is produced during chronic amphetamine administration, which directly or indirectly produces an increased sensitivity to amphetamine-induced stereotyped behavior. Lewander, however, found that amphetamine metabolites remain unchanged during chronic amphetamine administration in rats.[17,19]

The above discussion suggests that prolonged pretreatment with d-amphetamine produces a long-lasting hypersensitivity of striatal dopamine receptor sites to the action of subsequent dopamine agonists. Such a "chronic agonist-induced hypersensitivity" is a useful concept in understanding the enigmatic characteristics of levodopa-induced dyskinesias listed earlier.

Specifically, the linear relationship between duration of levodopa treatment for parkinsonism and the development of dyskinesias, the increasing severity and distribution of levodopa-induced dyskinesias, and the reappearance of these dyskinesias in spite of a decrease in levodopa dosage are various manifestations of a decreased threshold for the production of levodopa-induced dyskinesias. The shorter time between levodopa ingestion and the appearance of dyskinesias, of course, represents a decreased latency for the production of these dyskinesias.

An analogous decrease in the threshold and latency was found for the production of amphetamine-induced stereotyped behavior in guinea pigs during and following chronic d-amphetamine administsation as discussed earlier. It follows that a similar process of chronic agonist-induced dopamine receptor site hypersensitivity may underlie the development of hypersensitivity to amphetamine-induced stereotyped behavior in animals and hypersensitivity to levodopa-induced dyskinesias in humans. Further investigations of the effects of chronic dopaminergic agonism in animals promises additional insight into the nature of levodopa-induced dyskinesias in humans.

AMPHETAMINE-INDUCED DISORDERS

Amphetamine-induced psychosis is a well-recognized clinical entity which often mimics acute or chronic paranoid schizophrenia.[20-22,2] Chronic amphetamine use is also associated with dyskinesias which closely resemble movements seen in tardive dyskinesias, Huntington's chorea and a number of symptomatic choreas.[1] More complex stereotyped movements have also been reported in association with chronic amphetamine use. Coined "punding" by Rylander,[21] these movements characteristically involve such activity as the dismantling of mechanical objects, continual personal grooming and incessant tidying up.

A good deal of evidence linking amphetamine psychosis with cerebral dopamine activity has been summarized by Snyder.[2,12] The fact that amphetamine-induced dyskinesias and amphetamine-induced complex stereotypies are almost always reported in conjunction with am-

phetamine-induced psychosis[2] suggests that these disorders are also linked to cerebral dopamine activity.

Just as chronic amphetamine pretreatment produced a long-lasting hypersensitivity to subsequent dopamine agonism in guinea pigs a similar phenomenon has been reported in humans. Kramer[23] has noted that amphetamine psychosis does not usually occur early in the course of amphetamine abuse. With continued abuse the psychoses occur with decreasing latency and increasing intensity and may return rapidly with reinstatement of amphetamine use even after a long period of abstinence. Klawans and Weiner[24] found that a single small intravenous dose of amphetamine is sufficient to exacerbate chorea in patients with Huntington's chorea, Syndenham's chorea, or chorea associated with systemic lupus erythematosus, but not in normal controls. Each of these disorders is thought to involve physiologic alterations within the basal ganglia and an increased sensitivity to dopamine.[1] In view of the above experience, the fact that amphetamine does not cause paranoid psychosis or movement disorders early in the course of abuse but does so after chronic use,[24,25] suggests that chronic amphetamine intake produces a physiological change in dopamine receptor sites, resulting in an increased sensitivity to dopamine. The chronic agonist-induced hypersensitivity phenomenon observed in guinea pigs is also believed to be related to increased dopamine activity and may explain the development of dopamine hypersensitivity in chronic amphetamine abusers.

Griffith *et al.*[26] have confirmed the theory that chronic amphetamine use produces an alteration in the central nervous system, which lowers the threshold for specific amphetamine-induced behaviors. They report that chronic amphetamine abusers who show a tolerance to tyramine-induced increases in blood pressure usually present with psychoses closely resembling parnoid schizophrenia. Episodic amphetamine abusers intolerant to tyramine-induced increases in blood pressure commonly presented a more toxic psychosis, with disorientation and clouding of consciousness as a result of a single large dose of amphetamine.

It is interesting to note that psychoses and dyskinesias closely resembling those observed in chronic amphetamine abusers have been reported in association with chronic amphetamine and methylphenidate treatment in hyperactive children.[27-33] In several cases dyskinesias and psychoses did not develop in the children until several months of amphetamine administration. These symptoms remitted upon withdrawal of the amphetamine and returned abruptly with readministration of the drug. Thus a long-lasting hypersensitivity to amphetamine-induced disorders appears to be present in this population as well as in the chronic amphetamine abuser.

The appearance of such closely related symptoms in two diverse groups suggests that these symptoms are direct effects of chronic amphetamine use and are not due to latent psychotic traits or secondary drug effects. Furthermore, the chronic agonist-induced hypersensitivity model may be extended to account for dyskinesias and psychoses occurring in hyperkinetic children receiving chronic administration of dopamine agonists.

HYPERKINETIC SYNDROME

The term hyperkinetic syndrome has been commonly used to refer to a symptom complex occurring in children who demonstrate poor school performance, increased motor activity, decreased attention span and easy distractability. Some view this syndrome as a psychiatric disorder while others believe it the manifestation of an organic minimal brain dysfunction. Specific organic causes such as prenatal injury or hypoxia, other complications of pregnancy, neonatal febrile illnesses, encephalitis, and meningitis have been postulated but no consistent neuropathology has been identified in the hyperkinetic syndrome.

The fact that the most effective drugs

in the treatment of the hyperkinetic syndrome are central nervous system stimulants has been puzzling. A commonly cited explanation is that analeptic drugs act paradoxically in hyperkinetic children producing a decrease in central nervous system activity. While this is a convenient theory, the fact is that no such decrease in central nervous system activity has been well documented behaviorally or neurophysiologically. This inability to explain the effects of analeptic drugs on hyperkinetic children may result from a failure to differentiate adequately between disorders of motor activity and disorders of attention. While improvement of hyperkinesia with analeptics is often reported as a quantitative decrease in motor activity, studies utilizing mechanical devices or clinical ratings to measure general motor activity have not verified such a decrease with methylphenidate[34,35] or d-amphetamine.[36]

Prominent symptoms of the hyperkinetic syndrome are disorders of attention such as a shortened attention span, easy distractability and a decreased ability to focus attention. Studies utilizing vigilance tests and clinical ratings have confirmed that d-amphetamine[37,29] and methylphenidate[29] improve attention in hyperkinetic children. These facts suggest that an increased ability to focus attention, resulting in more adaptive motor activity may represent the real therapeutic effect of analeptic drugs in hyperkinetic children. The importance of attentional factors in the hyperkinetic syndrome is underscored by follow-up reports indicating that adolescents who were believed years earlier to be hyperkinetic showed improvement in terms of a decrease in excess motor activity but remained impaired in the spheres of attention and impulse control.[38,39]

Keeping in mind the importance of the attentional components of the hyperkinetic syndrome, an investigation of the pharmacology of this disorder is quite revealing. Methylphenidate (Ritalin®) and d-amphetamine (Dexedrine®) have been found to be the two most efficacious agents in treating the hyperkinetic syndrome.[40,41] Imipramine (Tofranil®) has also been found effective in reducing aggressiveness, hyperactivity, and inattentiveness in hyperkinetic children.[32] Methylphenidate exerts its central effects by releasing dopamine from cerebral dopamine neurons,[42] while d-amphetamine releases and inhibits the cellular reuptake of dopamine and norepinephrine.[16] Imipramine, a tricyclic antidepressant, blocks the neuronal reuptake of dopamine and norepinephrine as well as of serotonin. The action of these drugs suggests that alterations in dopamine and perhaps norepinephrine activity are involved in the treatment of the hyperkinetic syndrome.

Arnold *et al.*[43] have recently reported that d-amphetamine and l-amphetamine have similar efficacy in the treatment of the hyperkinetic syndrome. Considering that the two isomers have equal ability to release dopamine from dopaminergic neurons, but d-amphetamine is about 10 times more effective than *l*-amphetamine in releasing norepinephrine from norepinephrine neurons, Arnold *et al.* interpret the similar efficacy of d- and l-amphetamine in the treatment of hyperkinetic children as evidence for a dopaminergic rather than a noradrenergic influence in this disorder.

Amphetamine-induced stereotyped behavior in animals, a reflection of increased dopamine activity within the corpus striatum,[44,45] involves the repetition of discrete, species-specific behaviors. At sufficiently high doses of dopaminergic agonists, animals engage in these stereotyped behaviors to the exclusion of all other activities. Such stereotypy appears to require an intense focusing of attention.

As discussed earlier, similar amphetamine-induced stereotyped behaviors are seen in humans in whom they are also believed to be a reflection of abnormally increased dopamine activity. Similar stereotypies have been observed when levodopa is used in humans[4] and attributed to the activity of dopamine formed from the levodopa within the brain. Repetitive stereotyped movements and vocalizations are also observed in Guy de la Tourette's disease, a disorder thought to be related to

increased dopamine activity in the brain.[3]

Considering firstly the link between dopamine and increased focusing of attention as reflected in stereotyped behavior, and secondly, the improvement of hyperkinetic symptomatology with agents which increase dopamine activity, it may be postulated that the hyperkinetic syndrome, and especially those cases characterized predominantly by abnormalities of attention are related to decreased dopamine activity in the central nervous system. While stereotyped behavior is believed to be dopamine dependent, increased locomotor activity is mediated by norepinephrine.[12] It would thus appear that the inability to explain the therapeutic value of amphetamine and other central nervous system stimulants in treating hyperkinesia is due to a concentration on the noradrenergic effects of these drugs while, overlooking their dopaminergic effects. In accordance with this theory, chlorpromazine, a dopamine and noradrenaline blocking agent, has been found to decrease hyperactivity but not improve distractability in hyperactive children.[29]

Those children diagnosed as "hyperactive" represent an extremely heterogeneous population. Conceivably, hyperactivity in an individual may represent a primary motor disorder, an apparent hyperactivity which reflects a preponderance of non-focused activity and attention, or an agitated anxiety response secondary to inability to focus attention. In each case, a drug which would increase the child's ability to focus attention (d-amphetamine, methylphenidate) predictably may be effective in producing clinical improvements.

In view of this theory, it may prove fruitful to investigate the role of dopamine in the pathophysiology of the hyperkinetic syndrome. Therapeutic trials of levodopa and biochemical determinations of dopamine activity in these children are two directions which this investigation may follow.

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PERCUTANEOUS NEPHROSTOMY AND ANTEGRADE PYELOGRAPHY

Jerome Hoeksema
Suresh K. Patel

ABSTRACT. Percutaneous nephrostomy and antegrade pyelography was performed on eight patients. The procedure was used to demonstrate the upper collecting system in selected cases of hydronephrosis when, for various reasons, the diagnosis was incomplete or inconclusive by conventional methods of investigation (i.e. excretory urography, retrograde pyelography, etc.). It was also used to temporarily drain a hydronephrotic kidney when other methods of decompression were not technically possible or carried undue risk. We found this safe and simple procedure to be a reliable diagnostic and, in some cases, therapeutic tool.

Translumbar placement of a needle in the renal pelvis and subsequent injection of contrast material to demonstrate obstructive lesions in the urinary tract is more than twenty years old (Kapandji; 1949).[1] In 1955, Goodwin and Casey[2] reported fifty-five cases of antegrade pyelography* giving the procedure its first real clinical test. They later described the technique of percutaneous puncture of a hydronephrotic kidney and insertion of a plastic catheter for short-term drainage.[3]

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*This term was selected to describe the procedure as distinct from the already established "retrograde pyelography." Other authors have used the term "percutaneous pyelography."

INDICATIONS

Indications for the use of antegrade pyelography are: 1) when there is evidence of hydronephrosis (unilateral nonvisualization of a collecting system on excretory urogram with evidence of dilated calices) and retrograde pyelography fails to demonstrate the upper collecting systems (as in surgical ligature of the ureter, neoplastic processes, ureteroneocystostomy, etc.); 2) in cases of hydronephrosis after urinary diversion (ileal loop conduits, etc.); 3) in hydronephrosis in infancy[4] when the excretory urogram fails to render a definitive diagnosis and cystoscopy and retrograde ureteral catheterization are difficult (especially in young males where the penile urethra is small).

Indications for leaving a plastic drainage catheter in the renal pelvis for temporary decompression of an obstructed kidney are as follows: 1) when, owing to the patient's condition, immediate surgery is inadvisable (i.e. sepsis, uremia, etc.); 2) when evaluating the degree of irreversible renal damage present when determining whether nephrectomy or reconstructive surgery is necessary; 3) when draining an infected renal pelvis in an attempt to eradicate infection prior to surgery.

TECHNIQUE

Percutaneous puncture of the renal pelvis and insertion of plastic tubing is performed under fluorscopic or ultrasonic control.[5] When fluoroscopy is used, contrast material can be given intravenously to aid in delineating the collecting system. The patient is placed in a prone position, the skin swabbed with an antiseptic solution and sterile drapes applied. A local anesthetic is injected at the puncture site. An 18-gauge needle with an external plastic catheter sheath is then inserted perpendicular to the skin directly over the dilated pelvis and advanced slowly with continuous, gentle syringe aspiration. When urine is aspirated the plastic catheter sheath is advanced and the needle removed. Several milliliters of urine are removed for appropriate analyses and cultures, and an equal amount of contrast material is injected. At this time if catheter drainage of the kidney is desired, the catheter is sutured to the skin, connected to a closed drainage system, and a dressing applied.

SELECTED CASES

Case 1. A forty-four-year-old female presented with symptoms of fever, chills, oliguria and back pain. Between the ages of thirty-three and thirty-six she had had several operations for a benign disorder which resulted in a right nephrectomy and an ileal loop conduit draining the left ureter. An excretory urogram at the time of admission revealed pyelocaliectasis of the patient's remaining left kidney, but the nature and location of the obstruction could not be identified (Fig. 1). An ileostogram was performed. This demonstrated a mid-ureteral diverticulum and an intraluminal filling defect just distal to the diverticulum (Fig. 2). Because of the patient's continued oliguria and sepsis, it was elected to drain the kidney through a percutaneous nephrostomy and to perform surgery at a later date when the patient's condition stabilized. A percutaneous nephrostomy and antegrade pyelogram showed the ureteral filling defect to lie in the distal ureter and the 7-cm mid-uretral diverticulum was again demonstrated (Fig. 3). The nephrostomy catheter drained well for five days during which the patient became afebrile and free of other symptoms. On the fifth day the drainage stopped. A small amount of contrast injected through the nephrostomy tube disclosed that the catheter tip had slipped out of the renal pelvis. Since surgery was planned for the following day no attempt was made to reinsert the catheter. At operation, the ureteral diverticulum, and an inspissated mucous plug producing ureteral obstruction were removed.

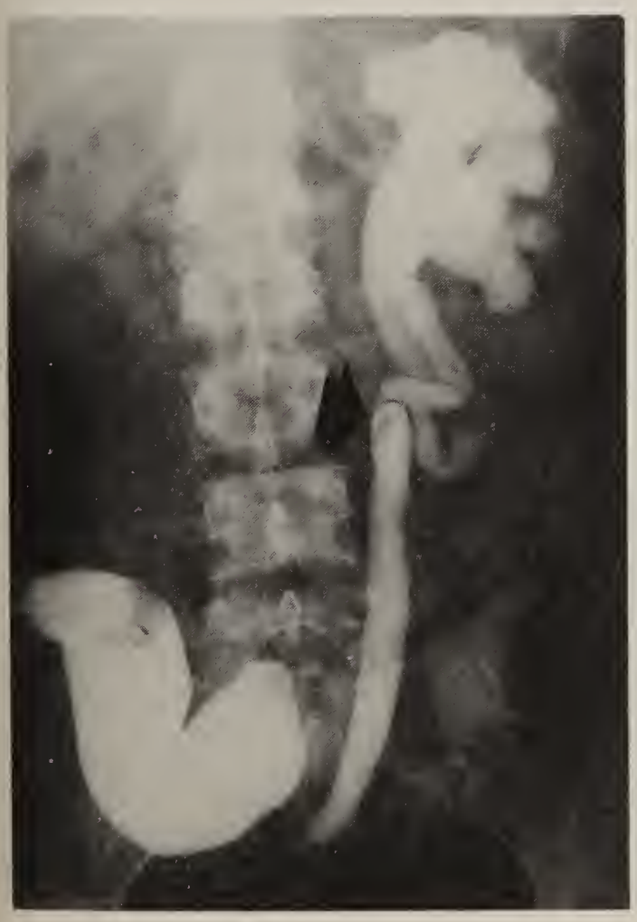
Case 2. A forty-nine-year-old female was admitted with right upper quadrant and flank pain, fever, and general malaise. Diagnostic studies revealed several gallstones, a polypoid lesion in the stomach, and a calculus in the right renal pelvis with non-visualization of the upper pole collecting system (Figs. 1 & 2). Cholecystectomy and partial gastric resection were performed. After the initial postoperative period she continued to have flank pain and fever. A right retrograde pyelogram showed non-filling of upper pole calices (Fig. 3). Selective renal arteriography demonstrated dilated upper pole calices without evidence of tumor vascularity (Fig. 4). A percutaneous nephrostomy tube was placed in a dilated right upper pole calyx (Fig. 5). Antegrade pyelography revealed a large dilated upper pole calyceal system with multiple granular filling defects. Thick yellow material was aspirated which, on culturing, revealed *B. proteus*. The tube was left in place, and the patient did well, her temperature returning to normal over the next several days. She subsequently underwent a right nephrectomy for xanthogranulomatous pyelonephritis.

CASE 1

Fig. 1—(Right) Excretory urogram demonstrating hydronephrosis.

Fig. 2—(Bottom left) Alleostogram. Solid black arrow marking intraluminal filling defect just distal to a ureteral diverticulum.

Fig. 3—(Bottom right) Antegrade pyelogram with small arrows marking ureteral diverticulum and filling defect now lying in the distal ureter.



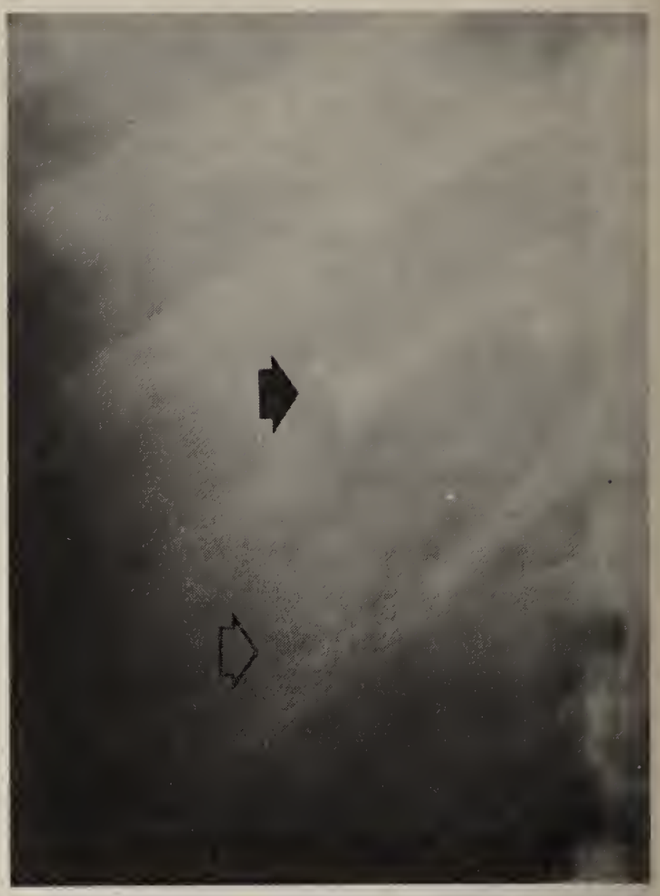
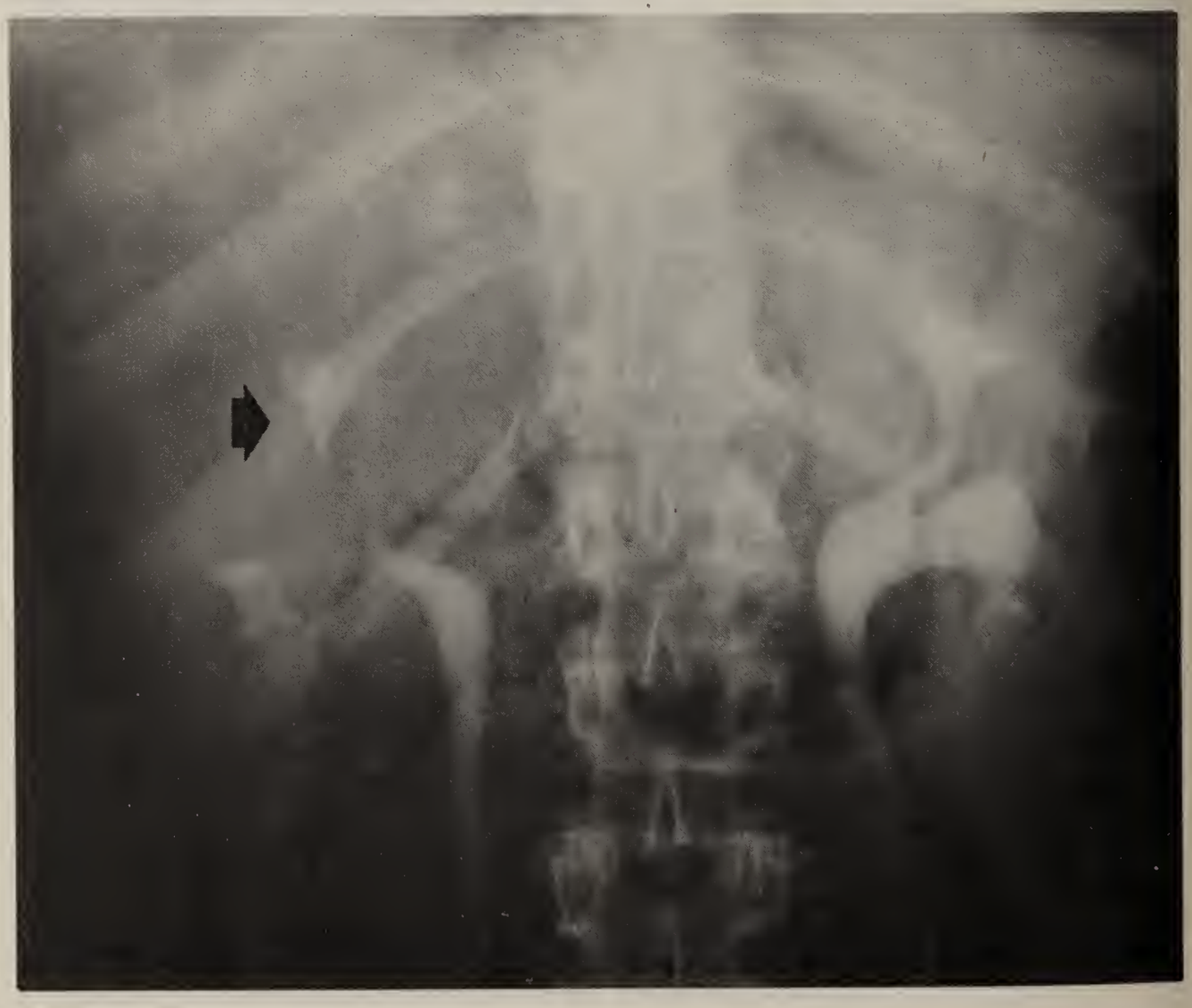
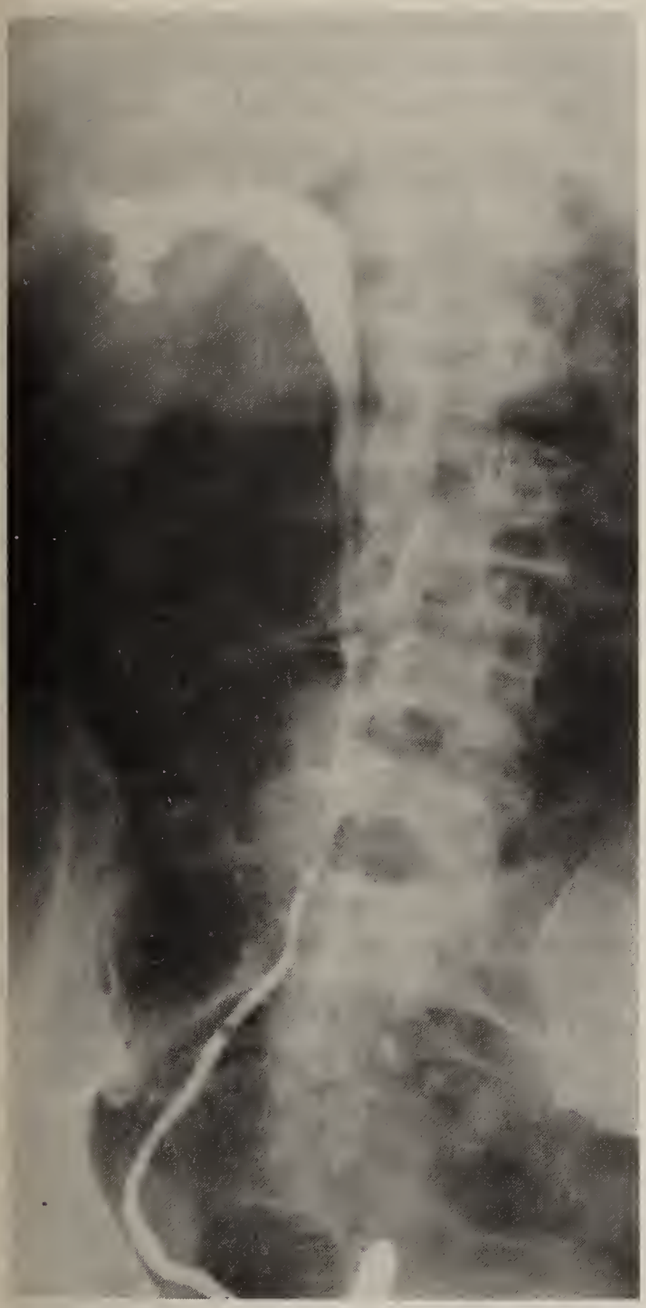


Fig. 4—(Right) Plain film of the abdomen demonstrates gallstones (open arrow) and renal calcifications (solid arrow).

Fig. 5 —(Below) Excretory urogram demonstrating intrapelvic calcifications marked by solid arrow and non-visualization of the right upper pole collecting system.





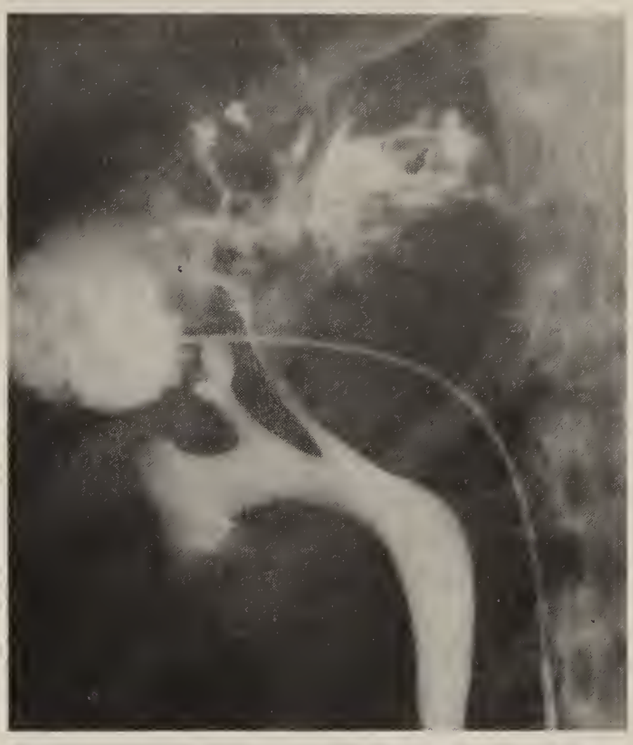
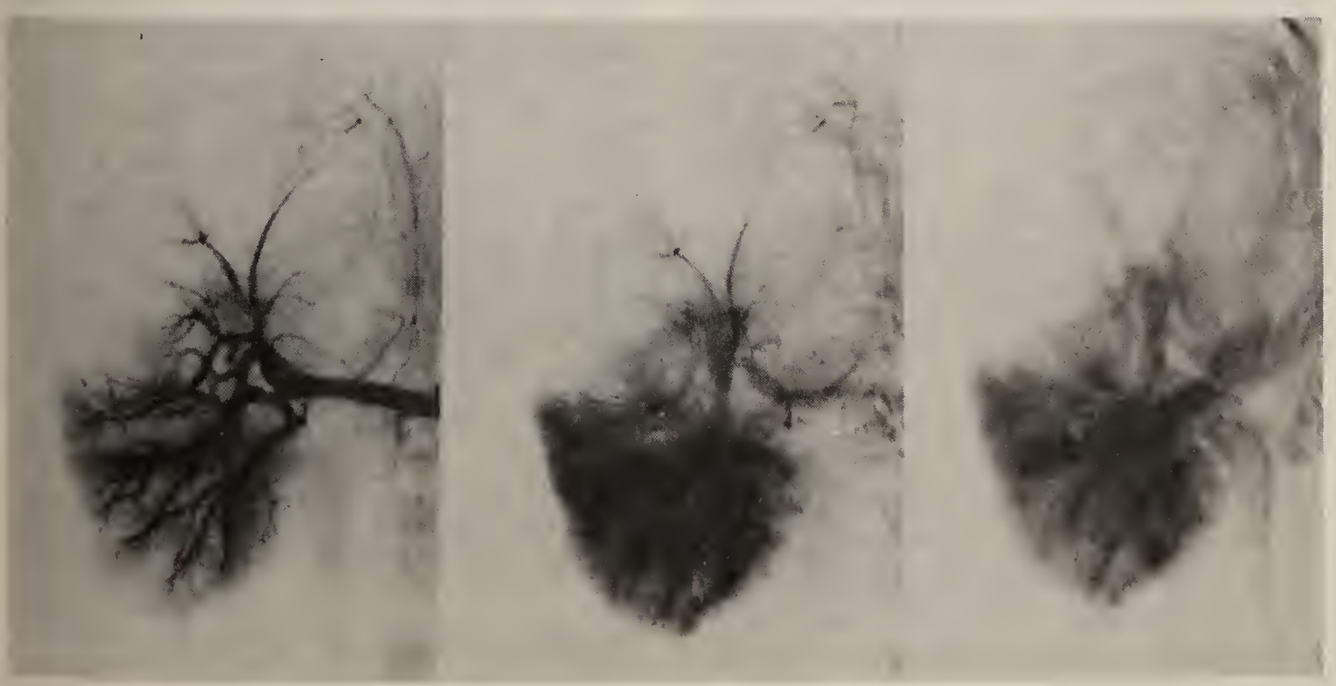


Fig. 6—(Left) Right retrograde pyelogram showing non-visualization of the upper pole.

Fig. 8—(Above) Right antegrade pyelogram showing contrast in dilated, incompletely filled, upper pole calices communicating with renal pelvis. Also noted are multiple, small, intraluminal filling defects.

Fig. 7—(Below) Selective right renal arteriogram showing localized hydronephrosis of the upper pole and no evidence of tumor vascularity.



DISCUSSION

Percutaneous nephrostomy and antegrade pyelography is not a new procedure. The procedure is simple to perform and requires no special equipment. We have used the technique successfully to aid in diagnosis and to assist in the management of eight patients, two of which are presented here. In our own experience and that of others, the procedure has been found to be safe but several complications should be considered: hemorrhage, infection, obstruction and kinking of the catheter.

Severe hemorrhage has not been encountered in our series. Anatomically, the kidney is the only vital structure lying between the skin and the renal pelvis. Arterial bleeding has been reported in the literature but has not resulted in serious complications.[3] Gross and microscopic bleeding is frequently encountered from a hydronephrotic kidney following decompression (either surgical or spontaneous). Blood-tinged urine draining from a percutaneous nephrostomy for several days following insertion has been seen in several of our patients but has been self-limited and devoid of serious complications.

Infection, probably, is the most serious complication. Adequate drainage after insertion of a nephrostomy tube in an infected renal pelvis is essential. If catheter drainage stops, the tube becomes kinked, obstructed or slips out of the renal pelvis, prompt action should be taken to determine the cause and, if necessary, a new nephrostomy tube inserted, or, as in Case 1, surgery should be performed to relieve the obstruction.

We emphasize that antegrade pyelography should be used only in selected cases where excretory urography and retrograde pyelography fail to render an adequate diagnosis and hydronephrosis is strongly suspected. Percutaneous catheter drainage of a hydronephrotic kidney should be limited to cases where immediate surgery is risky, or contraindicated, or when the determination of residual renal function is necessary for decision between nephrectomy or reconstructive surgery. In these particular cases antegrade pyelography and percutaneous nephrostomy can be safely used to supplement the diagnosis and to provide temporary drainage of an obstructed kidney.

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USE OF RADIATION THERAPY IN REVERSING THE ACUTE REJECTION PROCESS IN PATIENTS WITH RENAL TRANSPLANTS

Robert L. Spicer

ABSTRACT. Radiation therapy has been shown to be effective in reversing the acute rejection phenomenon in patients with renal transplants. The pathophysiologic interaction of radiation therapy with the immune system explains this success. The character of the rejection process as seen by its time of onset and by laboratory examinations of kidney functions may be important in predicting the outcome of radiation therapy. The nature of the treatment given with respect to number of fractions and cumulative dosage was not predictive of the end result.

INTRODUCTION

Twenty-nine patients who received radiation therapy at Rush-Presbyterian-St. Luke's Medical Center for threatened rejection of renal transplants were studied to determine the response of the rejection to irradiation. The parameters examined in this study are the onset of rejection, the response of renal functions to treatment, and the success or failure of the transplant.

THE IMMUNOLOGY OF REJECTION[7]

In transplantation of any kind, three major patterns or types of rejection may occur depending on the nature of the transplanted organ, the recipient, and their interactions as time progresses. The first type is the hyperacute rejection which occurs within minutes to hours after transplantation. This type of reaction is mediated by the recipient's preexisting humoral antibodies to antigens on the donor organ. It is felt that the recipient has been sensitized previously by a transfusion, pregnancy, cross-reacting bacterial infection, or by an earlier transplantation. The process which then takes place is that the antibody attaches to the endothelial cells of the transplanted organ, and complement fixation takes place. This leads to activation of the complement pathway, which in turn causes polymorphonuclear leukocytes to adhere to and injure the endothelial cell surfaces. Cellular damage initiates platelet attachment to the endothelial lining, finally resulting in thrombosis of the vessels and necrosis of renal parenchyma. It is an irreversible process, but is preventable by histocompatibility testing.

The second type of rejection, which is most important in this discussion, is potentially reversible with radiotherapy. The acute rejection, as it is called, usually occurs one week to four months post-transplantation. It is a classic type IV (cell-mediated) immune response. Thymus-derived T-lymphocytes circulate through the kidney and attach to cellular antigenic fragments which may be histocompatibility antigens or antigens created by injury to the kidney during transplantation. Cellular antigenic fragments can also enter the lymp system without lymphocyte attachment. These are carried to lymph nodes where they eventually come in contact with immunocompetent lymphocytes. This pathway from transplant to the lymph node is called the afferent limb of the immune response.

Once in the lymph node, the T-lymphocyte which has been sensitized by the antigen undergoes a series of cleavage

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divisions. As a result, plasma cells capable of antibody-production against the kidney, and transformed lymphocytes ("killer" cells or immuno-active cells) capable of destroying graft cells are formed. The killer cells then migrate to the transplantation site and initiate the destructive process. This is the efferent limb of the response.

Chronic rejection is the third type of reaction, and it is a two-fold process. Neither the acute or chronic rejection is as destructive as the hyperacute reaction, but if untreated they are equally irreversible. Non-complement fixing humoral antibody is formed, and it initiates an irritative proliferation of endothelial cells, gradually resulting in occlusion of the renal vascular supply. The second process is a membranous glomerulonephritis caused by antibody attachment to the epithelium above the glomerular basement membrane. Here, complement-activation causes glomerular basement-membrane damage leading to proteinuria. A spike-and-dome pattern of IgG immunofluorescence is seen extending from the epithelial cells to the basement membrane.

Only the acute rejection process is mediated directly by lymphocytes. The other types depend on lymphocyte production of antibodies, but the lymphocyte itself is not the effector. Radiation therapy's goal is to destroy or injure the lymphocytes mediating acute destruction of the transplant.

RADIATION TREATMENT: HOW IT IS USED AND WHY IT WORKS

Many experimental methods have been devised for prevention or arrest of the rejection phenomena.[2] Although usually ingenious, they have been impracticable, inherently dangerous, and generally unsuccessful in patients. Among these methods were total body irradiation, localized intravenous injection of radioactive isotopes, irradiation of the blood via intra-arterial implants or extra-corporeal shunting, and local dosages to the RE-system, thymus, and spleen. The only method of practical and popular use is local irradiation to the transplanted kidney. Its beneficial effects were first seen ten years ago by Myron Kauffman, at the University of Virginia, and have been borne out in experiments and clinical trials ever since.

Reasons for the effectiveness of radiation, although still somewhat a mystery, have been tested by animal experimentation. That radiation alters graft antigenicity has been disproved by the trials of Hume. Renal transplants were irradiated prior to transplantation, with the usual applied dosages (up to 1500 rads), and no change in survival from controls was noted.[2]

Alteration by radiation of local lymphatic structure such as drainage channels, nodes, etc. was thought to be a possible mechanism of action. But this is unlikely, since local lymphatics are not required for sensitization or destruction.[2]

Experimental use of radiation on kidney transplants in dogs has shown that radiotherapy affects both the afferent and efferent arcs of the immune response. All dogs in a studied group received a renal transplant. One-half of them were irradiated, one-half were not. After removal of the transplant and reimplantation of a second kidney, the dogs that received radiation were found to have a significant increase in survival time of the second kidney.[4] This indicates that the sensitization which would have been expected to occur, mediated by the first kidney, was blocked to some extent by irradiation. Interference with the efferent arc has also been shown to be important by Wolfe and Hume.[3] Dogs were given two renal homografts placed in different body locations, one in the neck and the other in the pelvis. One kidney was irradiated and the other was not. The kidneys which received radiation survived and functioned significantly longer than those which did not.

Finally, the non-specific, poorly understood, anti-inflammatory action of irradiation is given some credit for its effectiveness against rejection. Although the rela-

tive importance of each of these actions of irradiation is not known, that fact that radiation is *not* given to transplant padents until a rejection episode occurs (i.e., the recipient is already sensitized), indicates that radiation mainly blocks the efferent immune action and acts as an anti-inflammatory agent.

Deleterious effects of irradiation have been shown to exist, and include tubular necrosis.[5] However, a great advantage of radiotherapy lies in its absence of systemic side-effects.[6] Radiobiological studies indicate that renal irradiation has a very high therapeutic index. Radiation damage to renal architecture does not occur below doses of 2500 rads; whereas the small lymphocytes, the mediators of acute rejection, have a mean lethal rate of 100 rads.

CLINICAL STUDIES

The material for this study consisted of twenty-nine patients who received radiation therapy in an attempt to reverse episodes of rejection. Of these twenty-nine patients, fourteen had an unrelenting course resulting in removal of the grafted kidney (48 percent). Eight patients (27.5 percent) are alive, with a functioning kidney. Seven patients (24 percent) died of infections, emboli, or other causes not related to rejection.

The time of the first episode of rejection was studied to see if it was possible to predict when the rejection would occur, and to see if the time of the first rejection was predictive as to whether or not the kidney would be saved (Table I). The period from transplant to first rejection did not correlate with the eventual outcome of the transplant. The two patients who rejected on the 212th and 143rd days were both late rejections, and both kept their transplants (Table I).

For all patients the average number of treatments was 4.8. The fewest number of treatments given was one, and the most, ten, usually given every other day. Each dosage of radiation was 150 rads, making the average cumulative dose per patient 720 rads.

Analysis of treatment with respect to outcome is seen in Table II. Patients who rejected averaged two treatments and 255 rads more than those who did not. For whatever reasons, their course of rejection was more severe, thus requiring larger doses of radiation.

Measurements of blood urea nitrogen (BUN) and serum creatinine were made to see if the effect of irradiation on these critical indicators of renal function was uniform or predictive of the outcome of the transplant (Table III). Creatinine values appear to be more important than BUN in indicating a response to treatments. The average values of BUN were not significantly altered (62.1 to 62.2), but creatinine values fell from 4.0 to 3.5. An important point to realize, however, is that methyl prednisilone sodium succinate (Solu-Medrol®) is given concomitantly with radiation in rejection episodes. The administration of this steroid raises BUN

TABLE I

	Onset of Rejection (Days post-transplant)	Range of Rejection Onset (Days post-transplant)
Patients who eventually rejected	12	5-29
Patients alive with functioning transplant*	13	5-38
Patients dying of other cause	17	5-45

*This does not include those two patients who experienced their first rejection episode on days 143 and 212.

TABLE II

	Number of Treatments (Mean)	Cumulative Radiation Dosage (Mean)
All Patients	4.8	720 rads
Patients who eventually rejected	5.7	855 rads
Patients alive with functioning transplant	4	600 rads

TABLE III

ALL PATIENTS (MEAN VALUES)

	Day 1 of treatment	Day 1 after completion of treatment	Day 2 after completion of treatment	Day 3 after completion of treatment	Change from Day 1 of treatment to day 3 after completion of treatment
BUN	62.1	63.8	68.9	62.2	——
Creatinine	4.0	3.9	3.8	3.5	.5 (Decrease)

PATIENTS WHO EVENTUALLY REJECTED

	Day 1 of treatment	Day 3 after completion of treatment	Change from Day 1 of treatment to Day 3 post-treatment
BUN	68.4	86.5	18 (Increase)
Creatinine	4.9	4.8	.1 (Decrease)

PATIENTS ALIVE WITH FUNCTIONING TRANSPLANT

	Day 1 of treatment	Day 3 after completion of treatment	Change from Day 1 of treatment to Day 3 post-treatment
BUN	49.2	54.8	5.6 (Increase)
Creatinine	3.2	2.8	.4 (Decrease)

values in all patients, accounting for the increase over a period of time. When compared to those who rejected their transplant, patients who maintained their transplanted kidney had: (1) lower BUN and creatinine values at the start of treatment for rejection, and (2) better response by BUN and creatinine to the treatments. Those patients who eventually rejected their transplant may have had a more severe initial rejection episode causing a more rapidly progressing and less reversible kidney failure. They may have had a kidney which functioned less well even before rejecting, thus causing higher BUN and creatinine values. Possibly the treatment of the rejection episode was delayed allowing BUN and creatinine to reach higher levels and the rejection to progress until it was irreversible.

SUMMARY

Of twenty-nine patients treated at Rush-Presbyterian-St. Luke's Medical Center with radiation therapy in an attempt to reverse rejection of a renal transplant, 27 percent are alive, with a functioning kidney. No correlation was found between the time of onset of rejection and the failure or success of the transplant. The number of radiation treatments and dosages of irradiation were not related to the outcome of the transplants. Examination of BUN and serum creatinine is helpful not only in noting the onset of rejection, but in indicating the likelihood of response to radiation therapy. These values may also be used qualitatively to follow the reversal of the rejection episode.

ACKNOWLEDGEMENTS

The authors' gratitude for help in preparation of this paper goes to Drs. Frank R. Hendrickson and Myung-Sook Lee of the Radiation Therapy Department and Dr. Frederick K. Merkel and Ms. Sandra Seim of the Renal Transplant Section.

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BIOCHEMISTRY IN THE RUSSIAN SCIENTIFIC TRADITION

Anatoly Bezkorovainy

EDITOR's NOTE: This is the fifth in Dr. Bezkorovainy's series of articles on the history of medicine and allied sciences in Russia. The illustrations are reproduced from Russian postage stamps.

ABSTRACT. Biochemical research and teaching were conducted in Russian educational institutions well before World War I, though most Russian biochemists received post-doctoral training in German laboratories. The two major centers of biochemical research in Russia were the Dorpat University, and the St. Petersburg area. These were followed by Moscow University and Kazan University.

Oldest and most distinguished of the Dorpat biochemists was Carl Schmidt. He coined the term "carbohydrate," discovered the nature of amyloid, the common features among hexoses, and the nature of peptic digestion. Another Dorpat biochemist, Alexander Schmidt, formulated the modern theory of blood coagulation. Among contributors toward the understanding of the action of proteolytic enzymes, Pavlov's group and Danilevsky with his students and followers are the best known. Of the many botanists working in Russia at that time, Tsvet, the developer of chromatography, Ivanovsky, founder of the field of virology, and Winogradsky, who elucidated some nitrification and nitrogen-fixation processes, were the most distinguished. Investigators, who illuminated relationships between diet and disease included Anichkov and Khalatov, who implicated cholesterol in atherosclerosis, Kashin, the discover of the Kashin-Beck syndrome, and Lunin, the discover of vitamins.

INTRODUCTION

Biochemistry is a relatively young scientific discipline that emerged as a result of the efforts of diverse specialists in the physical and biological sciences. Most prominent among these were the representatives of older disciplines such as organic chemistry, physiology, botany, and microbiology.

In Russia, biochemistry followed this pattern of development. In addition, much support for biochemical and other basic scientific research came from the medical profession which, after the 1860's, came under strong influence of Rudolf Virchow's concept for a scientific basis in medical practice.[1] Thus, every medical school graduate who aspired to the degree of Doctor of Medicine, was expected to submit a thesis based on original research in one of the basic medical sciences. Such theses were generally of superb quality scientifically, and many described experiments that constituted fundamental advances in the basic medical sciences (e.g., Lunin's work on vitamins).[2]

The most influential Virchovian in Russia was Sergey P. Botkin (1832-1889), who spent his post-medical school years of 1856-1860 abroad working with Virchow, Traube and Hoppe. Upon his return to Russia, Botkin was appointed head of the medical clinic of the Military-Medical Academy in St. Petersburg, where he established a large laboratory for physicians working on doctoral research proj-

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ects. I. P. Pavlov did his doctoral thesis research in Botkin's laboratory. This laboratory later served as model for the Institute of Experimental Medicine in St. Petersburg. The medical establishment of the St. Petersburg area thus followed Botkin's ideas of a close integration of basic medical sciences with the clinical sciences.

A slightly different approach to medicine was followed by the Moscow school of medical practice headed primarily by Gregory A. Sakharin (1829-1898). Though he too for a time was a student of Virchow's, his approach to medicine, upon his return to Russia, was more empirical than scientific. Perhaps the influence of Inozemtsev (1802-1869), Sakharin's predecessor at Moscow University, was still too great to overcome. Inozemstev was a strong empiricist and disciple of the Viennese Rokitansky. During his tenure in the German laboratories, Sakharin discovered that potassium, and not sodium, was the principal cation inside the cells, and sodium was, therefore, primarily concentrated in the extracellular spaces.[3]

Though much of Russian biochemistry was imported from abroad, especially Germany, where future Russian professors of biochemistry (or medical chemistry) learned their trade from men like Hoppe, Abderhalden, Kühne, Hofmeister, and Kossel, there was one center in Russia where biochemistry developed independently: namely Dorpat University (at Dorpat, later Yuriev, later Tartu, now in the Estonian S.S.R.). Here biochemistry was in part an outgrowth of the famous Pharmacological Institute, developed by Rudolf Buchheim (1820-1879) during his teaching career at Dorpat (1846-1867).[4] Bidder, the Institute's professor, and Carl Schmidt (1822-1894), a chemistry professor, are considered to have established the Dorpat school of biochemistry.

Carl Schmidt was probably Russia's first biochemist to acquire international stature in his field. He was the inventor of the term "carbohydrate,"[5] discoverer of the nature of amyloid[6] and the nature of peptic digestion.[7] Unfortunately, his influence, as well as that of Dorpat's early school of biochemistry upon other Russian centers of learning, was not as great as it should have been. Dorpat was dominated by the Baltic German elite, and its students and professors preferred to take positions in Germany or Austria rather than in Russia. Hence, Russian universities had to look to Western Europe for inspiration in the field of biochemistry rather than to this first-rate establishment closer to home.

The Germanic orientation of Dorpat underwent a gradual change starting in the middle of the 19th century. Thus, Pirogov, the "father" of Russian surgery, was, in 1836, the first Russian to be admitted to Dorpat's faculty.[8] The integration of Dorpat with the rest of Russia's educational system became practically complete on the eve of the First World War, when the faculty consisted of as many Russians as Germans, if not more. As a result, there was a lively exchange of post-doctoral students and faculty members between Dorpat and other Russian universities, especially with universities in the St. Petersburg (now Leningrad) area.

CARL SCHMIDT, THE CO-FOUNDER OF THE DORPAT SCHOOL OF BIOCHEMISTRY

Carl Schmidt was born in Mitau, the capital of the Kurland province of Russia (now in the Latvian S.S.R.). He was educated at Dorpat, and remained on its faculty for some 40 years, beginning in 1846. He was interested in an amazingly large number of biochemical problems, including the chemistry of blood, the mechanisms of alcoholic fermentation and food digestion, and the chemistry of complex carbohydrates and proteins.

Schmidt is credited with the introduction of the term *carbohydrate (Kohlenhydrat)* into sugar chemistry. He used this term in conjunction with his extensive study of a number of neutral polysaccharides and simple sugars from plant sources, arriving at a general formula for

all neutral sugars, namely $C_{12}H_{10}O_{10}$.[5] Schmidt used atomic weights of 6 and 8 for carbon and oxygen respectively. In modern terms, the formula would read $C_6H_{10}O_5$, not too different from empirical formula of hexoses, $C_6H_{12}O_6$, or $C_6H_{10}O_5$ for glycosidically bonded hexoses.

The nature of amyloid, seen upon autopsy in many human organs, was elucidated by Schmidt. Up to that time it had been thought that the amyloid was a polysaccharide, hence the name *amyloid.* Schmidt was able to isolate very little carbohydrate from amyloid, but did find 15.6 percent nitrogen therein. From this he correctly concluded that amyloid was of protein nature, and that its name was a misnomer.[6] It is now known that amyloid fibrils consist of a number of proteins that include a glycoprotein, as well as immunoglobulin light chains.

The phenomenon of peptic digestion of proteins was well known among the early 19th century biologists, though the role of HCl was not completely appreciated. Schmidt's concept of peptic digestion of proteins involved the chemical combination of HCl with pepsin; HCl was constantly replenished as the digestion proceeded, and could not be replaced by another acid.[7] Though this theory was accepted for nearly 50 years, we know today that Schmidt was only partially right: instead of combining chemically with pepsin to form the active enzyme, HCl provides the acidic medium for peptic action, as well as activating pepsinogen into pepsin by the abstraction of a peptide.

Among other discoveries attributable to Schmidt's genius are his methods for the determination of uric acid in biological fluids, determination of blood volume, and his investigations on yeast metabolism. These will be dealt with at another time.

CARBOHYDRATE CHEMISTRY AND METABOLISM

Carl Schmidt was certainly one of the world's pioneering carbohydrate chemists; yet others in Russian laboratories made lasting contributions to this field. The first laboratory synthesis of a sugar was performed by A. M. Butlerov (1828-1886) (Fig. 1), an organic chemist and professor at Kazan and later St. Petersburg universities, using formaldehyde and alkali.[9] Formaldehyde was at that time a relatively new compound, having only recently been prepared from methylene iodide and silver oxalate by Butlerov himself, in the belief that he had actually synthesized methylene glycol. He visualized the synthesis of sugar as follows:

$$\underset{\substack{\text{methylene glycol}\\(\text{actually formaldehyde})}}{4\ C_2H_4O_2} \xrightarrow{\text{alkali}} \underset{\text{sugar}}{C_7H_{14}O_6} + \underset{\text{formic acid}}{CH_2O_2}$$

The product was sweet to the taste, was strongly reducing, but did not rotate plane-polarized light. Elemental analysis corresponded to a general formula of $C_nH_{2n}O_{n-1}$. Butlerov named the new compound *methylenitan,* and believed it to

Fig. 1—A. M. Butlerov, originator of the concept of the structure of organic compounds.

be similar to mannose. Butlerov's main contribution to science was, of course, his theory of the structure of organic compounds, which, with a few modifications, has so far withstood the test of time.[10]

The mechanism of glycolysis was under intensive investigation in a number of European laboratories before World War I, and there were several Russian investigators who contributed significant new knowledge to this field. Of great help in this regard were the botanists, such as Leonid Ivanov, co-discoverer of phosphorylation during glycolysis) who worked with yeast cells. This subject has been reviewed by the present author[11] and will not be reiterated in this paper.

PLANT BIOCHEMISTRY

Russia produced a number of other prominent botanists, many of whom, in addition to glycolysis, contributed significantly to other fields of the natural sciences. Most notable of these were Ivanovsky, founder of the field of virology, and Tsvet, the developer of chromatography. Many Russian botanists were interested in photosynthesis and the plant pigments; Russia of the pre-World War I era was one of the world's major centers of research in these areas. The contributions by Russian botanists to these fields are well summarized by Reed[12] and by Rabinovitch.[13] Other important botanists were A. N. Volkov (1849-1928) of Dorpat, who showed in 1866 that, in plants undergoing photosynthesis, the amounts of CO_2 utilized and O_2 produced were proportional to the light available; V. N. Lubimenko (1873-1937) of St. Petersburg, who discovered the relationship between the amount of chlorophyll present in a plant and the amount of light generally available to the plant; K. A. Timiryazev (1843-1920) of Moscow University, who, in 1868, proposed that chlorophyll was "sensitized" by light, thus converting solar energy to chemical energy; N. A. Monteverde (1856-1929), of the St. Petersburg Botanical Garden, who first studied chlorophyll transformation during photosynthesis; and Ivan Borodin (1847-1930), of St. Petersburg, who in 1883 first demonstrated the heterogeneity of yellow pigments in plants by crystallizing what were probably carotene and xanthophyll.

The most prominent Russian microbial biochemist, to receive his training as a botanist, was Sergei N. Winogradsky (1856-1953),[14] to whom Fruton and Simmonds attribute the "strengthening" of the tie between microbiology and biochemistry.[15] Winogradsky was director of the Institute of Experimental Medicine in St. Petersburg between 1891 and 1905, and was responsible for elucidating the actions of nitrifying and iron bacteria, and the nitrogen fixation process. He was responsible for isolating the first nitrogen-fixing organism, which he named *Claustridium pasteurianum.*

The Russian botanist best known to biochemists was undoubtedly Michail S. Tsvet (1872-1919), discoverer of chromatography. He obtained his education in Switzerland, worked in St. Petersburg from 1897 to 1902, and then moved to Warsaw University. Plentiful accounts of this remarkable man's exploits may be found in Western literature,[16-19] and will not be reiterated *in extenso* in this paper.

Tsvet's efforts were, of course, not aimed at developing the chromatographic method, but at separating plant pigments. Using sucrose or $CaCO_3$ columns, he was able to separate benzene, petroleum ether, CS_2, or CCl_4 extracts of plants into five components, identified as β-xantophyll, β-chlorophyll, α-chlorophyll, and two α-xanthophyll fractions. The carotenes were eluted in the void volume.[20] He also discovered the algal chlorphyll c, and pioneered the investigation of leucoanthocyanins.[21]

ENZYMOLOGY

The St. Petersburg school of physiological chemistry can trace its origins to Botkin's laboratories and later to the physiological investigations of Pavlov (Fig. 2) and his students. Understandably, such investigations were mostly concerned with

Fig. 2—Ivan P. Pavlov, in whose laboratories many biochemically-oriented projects were carried out. Pavlov won the Nobel prize for his work on digestive processes, and later established the field of conditioned reflexes.

the action of digestive enzymes on proteins and other food substances. Much of the work originating in Pavlov's laboratory was published in the form of doctoral theses or in Russian periodicals, so that secondary sources must be consulted in order to evaluate such contributions. A monograph by Babkin has, however, been most helpful in this respect.[22]

Among Pavlov's collaborators in the field of digestive enzymes were N. P. Shepovalnikov, the discoverer of enterokinase; Noll, who studied secretory granules of the salivary glands and the stomach wall; and Savich, Babkin, and Rubashkin, who investigated the secretory granules of the pancreas. These investigators showed that the secretory granules decreased in size following secretory activity of the pancreas, and eventually fused to form vacuoles. Babkin and Savich also showed that the secretion of protease, lipase, and amylase by the pancreas occurred in parallel rather than separately. This finding, along with that of Shepovalnikov, disposed of Pavlov's earlier theory to the effect that the pancreatic juice composition adapted itself to the nature of the food ingested. Babkin (b. 1877) was probably Pavlov's most distinguished pupil. He worked with Pavlov from 1902 to 1912, then moved to the Novo-Alexandriya Agricultural Academy, and later to Odessa. After World War I, he emigrated to Canada, where he spent many fruitful years with Dalhousie and McGill universities.

Another collaborator in Pavlov's laboratory, a Dr. Mett, devised a method for the measurement of proteolytic activity. This involved filling glass columns with solutions of eggwhite, heat-coagulating the eggwhite, and inserting the column into the enzyme solution to be tested. The activity of the enzyme solution was then expressed in terms of millimeters of the column liquefied per unit time.[23] The development of this method prompted Borisov, another co-worker of Pavlov's, to check exhaustively the previous finding of Schütz, who in 1885 had proposed that pepsin acted on proteins according to $v = k\sqrt{P}$, where v was the velocity of the reaction, P was pepsin concentration, and k was a constant. Borisov established an identical relationship for trypsin and amylase (using starch columns in the latter case), and found, furthermore, that at very high enzyme concentrations this relationship did not hold. For many years the above equation was known as the *Schütz-Borisov rule.*[24] The method of Mett, using the Schütz-Borisov rule for calculations, was a recommended procedure for measuring proteolysis in biochemistry laboratory manuals as late as 1948.[25]

Long before Pavlov's famous investigations on the function of the mammalian digestive organs which won him the Nobel prize, there was another Russian investigator, A. Y. Danilevsky (1838-1923), who discovered the multi-enzyme nature of pancreatic secretions and did fundamental work on the action of trypsin. Danilevsky is even credited by some with the discovery of trypsin,[26] though the latter claim is somewhat exaggerated. Before Danilevsky's investigations, it was

known that pancreatic juice caused the emulsification of fats, that it caused the inversion and degradation of sugar, and that it was probably responsible for the solubilization of coagulated protein (digestion). There was a controversy regarding the latter, since some investigators held that such solubilization was not due to the properties of the pancreatic juice, but to "putrefaction." Moreover, among those who agreed that pancreatic juice had the power to digest protein, there was a lack of consensus as to the optimal pH of the fermentation process. In addition, everyone agreed that all the enzymatic functions of the pancreatic juice were brought about by a single component called pancreatin, which was supposed to be a protein coagulable by heat and alcohol and in most respects similar to serum albumin and casein.

For his investigations, Danilevsky used both the natural pancreatic juice obtained from a canulated pancreatic duct of a large dog, and the aqueous extract of excised dog pancreas.[27] The pancreatic juice was mixed with a solution of collodion (soluble nitrocellulose), and separated into a precipitate and supernatant. The collodion was extracted from the precipitate with ether and alcohol, and the residue was dissolved in water. It was capable of solubilizing fibrin clots, but not of inverting sugar nor breaking down fats into glycerol and fatty acids. The reaction of solubilizing fibrin occurred in neutral and basic pH medium, but not in acidic medium. The activity was inhibited at 25° and 50°, and was abolished at 60°. Optimal activity was seen at 37 to 40°. The active material was always associated with protein material, but Danilevsky did not definitely state that the fibrin-solubilizing enzyme was indeed a protein. The active material did not give a xanthoproteic reaction, and it was precipitable with acid, though not with alkali.

The collodion supernatant was freed of ether and alcohol, and the precipitated collodion was removed by filtration. A large amount of inactive protein was precipitated from the supernatant by heating it to 43 to 44°, after which a large quantity of absolute ethanol was added. The sugar-inverting factor was in the alcohol precipitate, whence it was extracted by a 2:1 water-alcohol mixture. It inverted sugar in an alkaline medium and worked more slowly in an acidic environment. There was also a very small amount of fibrin-solubilizing activity in the invertase preparation, but no fat hydrolyzing activity. Danilevsky believed that the inverting factor was not a protein, since it did not give the xanthoproteic reaction, though he did feel that it was of a colloid nature, as was the fibrin-solubilizing factor.

Danilevsky was never able to isolate the fat-splitting factor from the pancreatic secretions. However, he did note that in preparing his pancreatic extracts, all endogenous fats were split into glycerol and fatty acids, giving an acidic character to the extract. When he treated the extract with MgO to remove the fatty acids, the lipid-splitting factor was lost, probably being absorbed into the MgO. Nevertheless, Danilevsky did believe that the lipid-splitting factor represented a third component of the pancreatic juice, the other two being the clot-solubilizing factor and the inverting factor. All three factors were, in Danilevsky's view, separate and independent enzymes with differing physical and chemical properties.

In 1886 Danilevsky discovered the phenomenon of plastein formation by gastric juice.[28] He believed that this represented a *de novo* synthesis of protein from peptone, and postulated the presence of a specific enzyme *chymosin* or *Labferment* to carry out this reaction. It was later found that pepsin was actually responsible for plastein formation. This reaction was investigated more fully by Zavyalov from Dorpat,[28] and it was found that the digests of many diverse proteins such as myosin, fibrin, and "Witte's peptone" could be converted into a gelatinous mass believed to represent new protein. Zavyalov believed in the so-called common core theory of protein structure, and the fact that a seemingly identical material (plas-

tein) was formed from such unrelated proteins as fibrin and myosin represented to him the correctness of this view. The plastein itself was studied by Lavrov from Dorpat,[29] who found that it gave the biuret, the Millon, and the xanthoproteic tests.

Danilevsky's work, in addition to its importance in understanding the function of pancreatic juice, also represented one of the first successful attempts to separate mixtures of enzymes. Danilevsky was certainly among the most gifted of Russian biochemists, and probably ranks on the same level with the famous Pavlov in terms of scientific skill and critical thinking. However, Danilevsky did not establish any "schools" of thought, nor was he able to gather a large group of students or followers. One reason for this perhaps was that his scientific activity (1860-1890) peaked during the years when science was not yet being so generously supported; another reason may have been Danilevsky's many moves during his most productive years. He graduated from the Kharkov University in 1860, then went abroad, and in 1863 was appointed to a professorship at Kazan University. Dismissed from this position in 1871 for having strong differences of opinion with the administration, he then went abroad once more to work with Virchow and other Western European luminaries. In 1885 we find him on the faculty of Kharkov University, and in 1892 he moved to the Military-Medical Academy in St. Petersburg. It is not known to what extent Danilevsky influenced the work of Pavlov, who was at that time also a member of the Academy's faculty.

Danilevsky's work apparently inspired another Russian investigator, Victor Pashutin, of the Military-Medical Academy of St. Petersburg, to improve upon the methods of separating the three types of enzymes.[30] He extracted pancreatic homogenates with sodium sulfite or ammonium nitrate to produce the protease (trypsin), with potassium arsenate to produce the carbohydrate hydrolase, and with potassium antimonate or sodium bicarbonate to produce the lipase.

The inactivation and reactivation of enzymes by heat was studied by M. J. Gramenitsky, who worked with N. Kravkov, the famous Russion pharmacologist (Fig. 3), at the Military-Medical Academy in St. Petersburg. Gramenitsky discovered that the substance taka-diastase (an enzyme which hydrolyzes starch) was lost when heated to 85 to 115°; then its activity regenerated if permitted to cool slowly to room temperature.[31] The hydrolytic activity of the enzyme disappeared at 70°, whereas its invertase activity was retained. In a similar experiment, if malt were heated to 100°, the maltase activity was irreversibly destroyed, whereas the amylase and oxidase activities could be recovered by slow cooling. In another investigation,[32] Gramenitsky permitted the taka-diatase, which had been heated to 85°, to recover its activity in neutral, acidic, and basic solutions. In the alkaline medium, activity was regenerated three times as rapidly as in water, and in an acidic medium the recovery of enzymatic

Fig. 3—N. P. Kravkov, a pharmacologist, whose associates carried out much important biochemical research. He also pioneered in the use of intravenous anesthesia during surgery.

activity was inhibited. Gramenitsky felt that the temperature sensitivities of enzymes could be used for enzyme purification. In view of our present-day knowledge of enzyme chamistry, it is clear that Gramenitsky's experiments had important implications in the understanding of the denaturation phenomenon as well as the three-dimensional aspects of protein structure. Gramenitsky, of course, did not recognize the significance of his findings, since at that time, there was no agreement regarding the chemical nature of enzymes, nor had it been definitely settled as to what really constituted a protein.

It is today well established that the process of blood coagulation is a series of enzymatic reactions. The founder of the modern theory of blood coagulation was Alexander Schmidt (1831-1894), a product of the Dorpat school of physiological chemisty. Alexander Schmidt was born on the island of Osel (presently in the Estonian S.S.R.), where his ancestors had migrated from Germany in search of religious freedom. Alexander Schmidt began his studies at Dorpat with the intention of following his family's wishes to enter the ministry; however, he soon changed his interests to medicine, and after additional studies abroad, was appointed to the faculty at Dorpat. From 1885 to 1889, he served as president of the University.[33]

Alexander Schmidt conceived his theory of blood coagulation in 1861 while working with Hoppe-Seyler in Virchow's laboratory in Berlin. His entire research career was devoted to amplifying and improving this theory, where coagulation was viewed as an enzymatic denaturation of fibrinogen by an active enzyme that arose from an inactive precursor.[34] Schmidt's theory assumed that essentially three substances were required for blood coagulation: 1) fibrinogen, which Schmidt callel *Fibrinoplastische Substanz;* 2) *Fibrinogene Substanz,* which was probably thromboplastin; and 3) a clotting enzyme *(das Fibrinferment),* recognized today as prothrombin or its active form, thrombin. Schmidt attempted to purify all three substances, with varying degrees of success. The fibrinogen was obtained from plasma by acidification with either dilute acetic acid, or by the passage of CO_2 through plasma.

The apparent thromboplastin was prepared from the pericardial fluid of horses by acidification or by saturating it with NaCl. Schmidt could not characterize it extensively because of its small yield. The clotting enzyme was, according to him, present in blood in an inactive form, and was, to a large extent, co-precipitated with the fibrinogen. It was supposed to be activated by O_2 in the air.

That blood coagulation was indeed an enzymatic process was shown by Schmidt via the following observations: 1) very small amounts of the purified enzyme were sufficient to initiate clotting of fibrinogen; 2) boiling the enzyme preparation destroyed its activity, and it was very weakly active at 0°C. Its maximum activity was observed at 45°C; and 3) it was not destroyed during clotting, since the supernatant of the fibrin clot could initiate clotting in a fresh system. The purified enzyme was prepared by diluting plasma with 15 to 20 parts of "strong" alcohol, and permitting the precipitate to stand no less than 14 days. The precipitate was then extracted with water to obtain the active enzyme.

THE STRUCTURE AND METABOLISM OF PROTEINS AND AMINO ACIDS

A protein, at the turn of the century, was considered to represent a substance which contained nitrogen, gave biuret, Millon, and xanthoproteic reactions, and was coagulable by heat. Most people also recognized that amino acids were the sometime components of proteins. Much information on protein structure was gathered on the basis of peptic or tryptic digestion, and, since at that time very little was known about the specificity of these enzymes, erroneous conclusions were often reached on the structure of proteins.

Such experiments, however, when properly carried out and interpreted, frequently yielded valuable information. A favorite protein investigated in many laboratories was casein, because it was readily available in quantity, and was considered by most investigators to be homogeneous. This view persisted well into the 20th century, when the technique of moving boundary electrophoresis showed that casein was in reality a mixture of at least four components.

One of the first Russian chemists to investigate casein was N. N. Lyubavin (1845-1918), who was initially a professor in St. Petersburg, and then taught in Moscow (1886-1906). In 1871, he published a paper reporting the results of investigations while on a leave of absence in Hoppe-Seyler's laboratory.[35] Lyubavin was able to isolate a phosphorus-containing insoluble fraction, and a water soluble fraction from the digest. From the latter, he crystallized leucine and tyrosine, and showed the presence of peptone therein. This work is remembered by some historians as conclusive evidence for the existence of amino acids in proteins. In addition to isolating the two amino acids. Lyubavin also communicated his thoughts on the nature of protein digestion by enzymes. It had been noted for a long time that digested or hydrolyzed proteins contained more oxygen than the undigested proteins, and this prompted Mulder, father of the term *protein,* to propose that digestion is an oxidative process.[36] Lyubavin felt that this was not the case, and that, instead, protein digestion represented hydrolysis, i.e., the reaction of proteins with water. He proposed that the breakdown of proteins by acids, alkalis, water above 100°, enzymes, and bacteria (putrefication or "Fäulniss") were identical processes in this respect. A significant increase in the hydrogen content of digested proteins had not been noticed, according to Lyubavin, because such an increase, had it taken place, could not be detected by the methods then in use. After his return to Russia, Lyubavin attempted to prove his thesis that casein was simply a phosphoprotein, rather than a nucleoprotein, as had been previously postulated by several investigators, because of the casein's phosphorus content. Though he was, in retrospect, correct, he was not then successful in providing such proof. He did show that the phosphorus-containing residue obtained after peptic digestion of casein ("nuclein") was a heterogeneous substance, since "nuclein" preparations with different phosphorus content could be obtained from different batches of casein.[37]

One of the first investigators to suggest that casein was not a homogeneous protein was A. Danilevsky (see above). He separated casein into two fractions on the basis of solubility.[38,39] The casein was extracted with hot, 40 to 50% alcohol, yielding a precipitate called *Caseoalbumin,* and a soluble fraction called *Caseoprotalbstoff.* The former, Danilevsky believed, was similar to coagulated serum albumin. He later changed the terminology to *Nucleoalbumin* and *Nucleoprotalbstoff,* respectively, since both fractions contained phosphorus, and phosphorus, as stated above, was supposed to come from nucleic acids only. Danilevsky further believed that the two components were interconvertible, a conclusion he reached by comparing casein prepared by acid precipitation and that obtained from the action of rennin. Thus, whereas acid casein required a large quantity of alkali for neutralization, the rennin-coagulated casein required very little. Moreover, when the acid casein was extracted with the hot alcohol, a large portion of it was present in solution *(Nucleoprotalbstoff* fraction). On the other hand, very little *Nucleoprotalbstoff* could be obtained from the rennin casein. He therefore felt that the rennin was active against the *Nucleoprotalbstoff* only, converting it into *"Nucleoalbumin."* This action of rennin, in Danilevsky's view, involved the attachment of calcium ions onto the phosphate groups of the *Nucleoprotalbstoff.* Danilevsky's *Nucleoprotalbstoff* was probably what we recognize today as kappa-casein, a glycoprotein which stabilizes alpha-

casein, the major fraction of total milk casein. The action of rennin is now known to affect the splitting of kappa-casein into a glycomacropeptide and the para-kappa-casein, thus destabilizing and causing the precipitation of alpha-casein. Acid casein is known to contain kappa-casein in the unaltered state. Needless to say, Danilevsky's views were severely criticized in the biochemical literature, and were soon all but forgotten.

Another Russian investigator who attempted to elucidate the specificity of proteolytic digestion and, at the same time, the structure of proteins, was D. Lavrov, who first worked with Danilevsky at the Military-Medical Academy in St. Petersburg, later moving to Dorpat. He digested fibrin with pepsin, and the digest was then fractionated with ammonium sulfate and alcohol. There were six fractions with varying ammonium sulfate precipitability and six fractions not precipitated by ammonium sulfate, but precipitable with alcohol. The various fractions were quite different with respect to optical rotation, the biuret and other typical protein color reactions, and precipitability with acids. The more soluble the fraction was in water, the fewer protein-positive reactions it tended to give. Extended digestion with large amounts of pepsin yielded products that gave no reactions typical of proteins. Tryptic digestion yielded essentially similar results.[40] In a later investigation, Lavrov was able to isolate crystalline leucine, valine, and aspartic acid after prolonged (8 weeks) digestion of proteins. In addition, he found cadaverine and putrescine in such digests, which, he felt, were produced from lysine and arginine by peptic digestion.[41] More likely, these products were produced via bacterial contamination of the digest, though Lavrov claimed that his reaction mixtures were sterile. Lavrov and his students later developed the theory that the true function of pepsin was to hasten the hydrolysis of proteins by the HCl present in the stomach. One set of experiments supporting this hypothesis was provided by E. Svirlovsky, a collaborator of Lavrov at Dorpat, who subjected a number of proteins (hemoglobin, casein, albumin, gelatin) to several months' hydrolysis with 0.5 percent HCl at 36 to 37°.[42] He was able to isolate free amino acids, such as histidine and arginine, from all proteins except gelatins. Also isolated were other products, which, upon hydrolysis with 20 percent H_2SO_4 at 100° for six hours yielded free amino acids. Similar results were, of course, obtained by the peptic digestion of proteins. Lavrov and his students were thus among the first to suggest that peptides were products of proteolytic digestion of proteins.

Of the more exotic nitrogenous compounds, associated with discoveries of Russian biochemists are carnosine, carnitine, and spermine. Carnosine and carnitine were discovered and their structures defined in Vladimir Gulevich's laboratory at Moscow University. S. Amiradzibi and R. Krimberg were his most prominent associates in this venture. These investigations have recently been described in detail.[43]

A substance that was reputed to cure various kinds of diseases was isolated from mammalian testes by v. Poehl and named spermine.[44] Von Poehl (1850-1908) was a professor first at Dorpat, then in St. Petersburg. He prepared spermine as a platinum hydrochloride complex, whose empirical formula was $C_5H_{15}N_2PtCl_6$. The free base was assigned the formula $C_5H_{13}N_2$. Its therapeutic effects were eventually proven to be nil, and its physiological function remains as yet unknown, though there is evidence that spermine regulates glycoprotein biosynthesis.

The isolation of new proteins was not as commonplace at the turn of the century as it is today, when almost every issue of a biochemical publication contains a description of a purified enzyme or another biologically active protein. Among the few that had been purified by the end of the 19th century was a group of basic proteins called protamines, generally found in the testes of fish. One such protein was isolated by D. Kuraev (1869-1908) from the testes of the Baltic Sea mackerel.[45] It

contained 24 percent nitrogen, about 21 percent of sulfate, and gave an empirical formula of $C_{30}H_{60}N_{16}O_6 \cdot 2\,H_2SO_4$. Kuraev called the new protein *scombrine sulfate,* a name still used. The protein was very basic, gave a strong biuret reaction, and had a specific rotation of −72°. Histidine and arginine were found in its sulfuric acid hydrolysates.

Kuraev also proposed a then novel method for the determination of the molecular weights of proteins. He iodinated crystalline serum and egg albumin, and found preparations that contained as much as 10 to 12 percent iodine.[46] He assumed that the iodine reacted with the nitrogen atoms of proteins, and estimated that in his serum albumin there were 11 iodine atoms for every 116 nitrogen atoms. From this he deduced an empirical formula for serum albumin, $C_{450}H_{693}I_{11}N_{116}O_{132}S_4$, which gave a molecular weight of 10,000 to 11,000. This was higher than most such values theretofore suggested for serum albumin, but was, of course, far short of the 65,000 to 70,000 value that is the true molecular weight of albumin. Today, Kuraev's approach to the determination of molecular weights of proteins can be recognized in any of the number-average molecular weight determinations, e.g., the method of end-group analysis.

Among the most influential biochemists in Russia as well as in all Europe was Marcel v. Nencki (1847-1901), a Pole by nationality, who studied in Cracow, Berlin, and Jena, worked for many years in Switzerland, and who in 1891 was appointed as head of the physiological chemistry laboratories of the newly-opened Institute of Experimental Medicine in St. Petersburg.[47] To Nencki is attributed much of the classical work on hemoglobin and heme compounds, and he probably introduced this field of research into Russia. Nencki's biography has been recently published in the West.[48]

NITROGEN METABOLISM

Marcel Nencki was also a pioneer in attempts to clarify the formation of urea in mammals, and much of this work was done in Russia. As early as 1869, Nencki and Schultzen reported that urea was capable of being formed from glycine.[49] In dogs on low protein diets, urea excretion dropped to 4 to 5 g/24 hours. When acetamide was fed, the output immediately increased to 15 g. The additional nitrogen excreted corresponded to the amount of acetamide fed. The same finding was obtained with glycine: an equivalent of 12 g of urea in glycine was fed, and 10 to 11 g were excreted. With leucine, a urea equivalent of 9.3 g was fed, and 8 g were excreted. More sophisticated experiments were later performed by Sergey Salaskin, who perfused dog livers with blood containing added glycine, aspartic acid, and leucine. In all cases an increase in blood urea was obtained, with 100 percent of the glycine and leucine being converted into urea.[50] This not only furnished conclusive evidence that urea is formed from amino acids, but also implicated the liver in the process.

The implication of ammonia in the formation of urea was suggested by Nencki in collaboration with Pavlov and others.[51] It was found that dogs with the "Eck fistula" (devised by N. Ekk from the Military-Medical Academy in St. Petersburg),[52] or cavo-portal shunt, could not tolerate meat and would exhibit acute toxic effects, primarily in the nervous system, when fed nitrogenous compounds. It was also found that the urine of such animals contained large amounts of what was thought to be carbamic acid, and the toxic symptoms seen in dogs with the Eck fistula could be reproduced in healthy dogs by the injection of carbamic acid. (It is not clear exactly what was meant by "carbamic acid," since free carbamic acid is unknown; it is possible that acidified ammonium carbamate served as a source of "carbamic acid.") A number of organs were then analyzed for the presence of ammonia, which was found to predominate in the lining of the stomach and the intestine. The ammonia content of the portal vein was high compared to the blood of other vessels: 3.5 to 8.4 mg/

100 g blood in the portal vein *vs.* 0.5 to 1.8 mg/100 g in the hepatic vein. The urea content of both veins was almost identical. The ammonia content of arterial blood was 1.5 mg/100 g; however, in animals with the Eck fistula the ammonia level reached 5.4 mg/100 g, i.e., a value identical to that of the portal vein. It was concluded that the ammonia, probably in the form of ammonium carbamate, was transported via the portal vein from the gastrointestinal tract to the liver, where it was converted into urea. The liver was thus assigned the role of a detoxification device.[53] It was also suggested that uremia was nothing but an accumulation of ammonia in the blood due to the failure of some sort of detoxification mechanism. However, Salaskin was not able to demonstrate the presence of excess ammonia in uremic dogs whose ureters had been tied, nor the presence of abnormal amounts of ammonia in the brains of patients who had died of uremia.[54] Salaskin had previously found that dogs with the Eck fistula had high accumulations of ammonia in all the tissues, especially that of the brain. This work thus demonstrated that uremia was not due to a failure of the organism to convert ammonia to urea.

To add a postscript to the section on protein chemistry and metabolism, one can jump ahead some 50 years and contemplate the recent successes of molecular biology in elucidating the genetic code and the mechanisms of protein synthesis. A great stimulus for these discoveries was the work on bacteriophages, i.e., viruses, conducted in the 1950's and 1960's by Hershey, Kozloff, and others. The existence of viruses was, of course, discovered by a Russian botanist, Dimitry Ivanovsky (1864-1920), a professor of St. Petersburg and later of Warsaw Universities (Fig. 4). In his investigations on the tobacco mosaic disease,[55] he found that the agent which transmitted the disease from one plant to another was filterable through a bacterial filter, but was destroyed upon boiling. He concluded that the disease was caused by a very small microbe which we today call the tobacco-mosaic virus.

Fig. 4—Dimitry Ivanovsky, the discoverer of the tobacco mosaic virus.

ENDOCRINOLOGY

It is generally believed that modern endocrinology was born with the discovery of secretin in 1902 by Bayliss and Starling, who also proposed that hormones were substances produced in one organ to exert their effects elsewhere in the body. Secretin could very easily have been discovered in Pavlov's laboratory in St. Petersburg, and Pavlov and his students were indeed ridiculously close to its discovery. However, Pavlov's group, as well as most other Russian physiologists, were under the overwhelming influence of Sechenov, the "father" of Russian physiology, who believed in an almost absolute domination of the human organism by the nervous system. This rigidity of thought is well demonstrated by the work of Popielski, a student of Pavlov, and later the director of the Pharmacological Institute of Lemberg (Austrian Poland). Popielski, while in Pavlov's laboratory, was studying the mechanism of pancreatic secretion elicited by the introduction of 0.5 percent HCl into a duodenal fistula.[56] Pancreatic

secretion was studied after cutting the spinal cord, the vagi, and the splanchnics. Then, after destruction of the spinal cord and medulla (dogs were kept under anesthesia, with an artificial respirator). Pancreatic secretion still persisted. He then cut the stomach as well as the small intestine at several locations thinking that a local nervous network might be involved, but this was without effect on pancreatic secretion. The celiac plexus was then destroyed, also without effect. Popielski thus all but eliminated the nervous control of pancreatic secretion, yet he did not perceive this, and proposed instead that the pancreatic secretion was controlled by an as-yet-unrecognized nerve center. Even after publication of the work of Bayliss and Starling, Popielski refused to believe that secretin played a major role in pancreatic secretion.[57]

The presence of endocrine glands in the mammalian organism was known in the 19th century. However, their function was not clarified until after the discovery of secretin by Bayliss and Starling. Research on the role of the endocrine glands was largely limited to studying their composition and to the physiological effects of their extracts. Such physiological effects, e.g., changes in blood pressure, were interpreted in various ways, and Russian investigators made such interpretations invariably in terms of changes in the nervous system activity (e.g., Cyon's view on the function of thyroxin). The situation did not improve even after Baumann's discovery of the role of iodine in the function of the thyroid gland.

The first investigator to study the composition of the thyroid gland was N. Bubnov (1851-1884), a student of Botkin at the Military-Medical Academy in St. Petersburg and a classmate of Pavlov's. He participated in the Russo-Turkish war of 1876-78 under Pirogov (the father of Russian surgery), then continued working with Botkin until he contracted diphtheria from a patient, and died. Bubnov was particularly interested in the nature of the "colloid" substances from the thyroid glands.[58] He extracted both the bovine and human glands first with water, then with 10 percent NaCl, and finally with two portions of 0.1 percent KOH. The water extract contained mostly small molecules such as lactic acid, hypoxanthine, and guanine, although it also gave a positive test for proteins. The next three fractions consisted of proteins, which Bubnov called *Thyroprotin*. The proteins were precipitated from the 10 percent NaCl and the two KOH extracts with dilute acetic acid, and represented what Bubnov felt were homogeneous thyroproteins 1, 2, and 3. The bovine thyroproteins gave ash values of 1.4, 1.7, and 1.5 percent, respectively, 16.0, 16.1, and 16.7 percent nitrogen respectively, and 49.4, 50.2, and 49.3 percent carbon respectively. The human materials gave 1.7, 1.3, and 1.6 percent ash, 15.9, 15.8, and 16.7 percent nitrogen, and 49.5, 50.3, and 49.2 percent carbon, respectively. Bubnov did not do iodine analyses of his protein fractions. His work is considered to have laid the groundwork for the future isolation of thyroglobulin, a large protein from the thyroid glands, which is intimately involved in the synthesis of thyroxine from tyrosine. The thyroglobulin was most likely present in Bubnov's 10 percent NaCl extract of the thyroid glands (thyroprotein 1).

Of the Russian biochemistry researchers who published in the field of endocrinology, the name of Elie de Cyon is probably most outstanding. He sought to unravel the functions of the thyroid, pituitary, and adrenal glands, but his explanations were invariably based on the actions of the nervous system. De Cyon's interesting conclusions are described in detail elsewhere.[59]

NUTRITION AND THE BIOCHEMICAL BASIS OF DISEASE

Russia's Dorpat University was one of the world's major centers of nutritional research. Here Georg Bunge (1803-1897) was the main force behind this approach to biochemistry. One of the many postdoctoral researchers whom Bunge attracted was N. Lunin (1854-1937) of St. Pe-

tersburg, who, at the suggestion of Bunge, attempted to evaluate the significance of common salts in the nutrition of animals. Instead, Lunin discovered the existence of other indispensable nutritional factors which we today call the vitamins.[2] This was some 25 years before Hopkins' similar observations in 1906. Lunin's work is summarized in a recent publication by Bezkorovainy.[60]

Russian medical investigators established the relationship between high fat diet and circulatory disorders. As early as 1868, V. C. Krylov, working in Rudnev's laboratory at the Military-Medical Academy in St. Petersburg, reported a correlation between fat content of the heart and heart "degeneration."[61] In 1909, Ignatovsky reported that rabbits developed damage to the liver and aorta if they were maintained on animal diets (meat, eggs, milk) as opposed to vegetable-based diets.[62] Ignatovsky attributed these effects to animal protein. In 1912, Stuckey excluded protein as the causative agent, since feeding of egg yolk did not produce the same effect as egg white. He proposed fat as the toxic agent. However, the maintenance of rabbits on diets rich in neutral fat did not produce the expected pathology. Vaselkin's experiments later excluded lecithin.

It was therefore only a matter of time until cholesterol became implicated, this discovery occurring in 1913 through the efforts of Anichkov and Khalatov. Anichkov (b. 1885) was a graduate of and later professor at the Military-Medical Academy in St. Petersburg; Khalatov (b. 1884) was appointed professor of pathology at Moscow University after his tenure at the Military-Medical Academy from 1908 to 1922. In their initial paper[63] Anichkov and Khalatov reported the results of feeding pure cholesterol to rabbits. In four to eight weeks, they noted fat droplets in the liver parenchyma, spleen, and aorta. The pathology was identical with that seen by Stuckey in his egg-yolk experiments. Blood cholesterol values were also very high in these animals. Although guinea pigs reacted like rabbits, rats were, on the other hand, immune to tissue fat infiltration. In a continuation of this work,[64] Anichkov made extensive histological studies on the affected tissues. He noted tissue infiltration by fat in the form of cholesterol ester "liquid crystals," and observed that such "crystals" caused cell destruction in the liver and formation of scar tissue in its place. In the aorta, fat droplets accumulated in the extracellular areas, localizing along the elastin fibers. The latter were eventually altered into a fine mesh-work, which entrapped phagocytes. The middle and inner layers of the aortic wall became indistinguishable; aortic muscle fibers were also damaged. Anichkov noted that the histologic picture seen in rabbit aortas was similar to that seen in aortas of sclerotic human beings, and proposed that this disorder was caused in humans by excessive amounts of cholesterol in the diet.

An interesting iron-intoxication disease, similar to hemochromatosis, was described in 1861 by N. Kashin (b. 1825), a medical officer in Siberia. According to Schipatschoff,[65] Kashin observed a disease endemic to a 20,000 km^2 area in an isolated Siberian area called the Urow Valley. The disease already began *in utero*, and progressed through rickets, scurvy, polyneuritis, and osteoarthritis. The bones of the affected individuals were so deformed and their muscles so atrophied, that they were complete invalids by age 40. Kashin believed that the drinking water was responsible for the disease, and by moving the inhabitants of that area elsewhere it was possible to arrest or cure the disease. He proposed *en-masse* resettlement of the population to a nearby location, and this was apparently partially carried out. In 1906, Beck, another Russian investigator, confirmed Kashin's observations, but concluded that the disease was primarily of osteoarthritic origin. In 1910, Vel'yaminov proposed that the disease was basically of thyrotoxic origin, since the thyroid was enlarged in most of the afflicted individuals. He also termed the ailment the *Kashin-Beck syndrome*. In 1931, Schipatschoff proposed that the

Kashin-Beck syndrome was of the vitamin-deficiency variety, since bread baked from crops in the affected area, when fed to laboratory animals, brought about symptoms of the disease.

In 1939, a Japanese investigator, K. Hiyeda, proposed that the Kashin-Beck syndrome was caused by iron intoxication.[66] The disease was found to be widespread among certain population groups of Manchuria, then under Japanese occupation. Hiyeda found that well water in the affected areas contained more than 0.3 mg of iron per liter, with frequent readings of 10 mg/1. Foods grown in the area also contained high amounts of iron. Histologic examination of tissues obtained from those suffering from the disease showed large accumulations of iron granules. Hiyeda suggested, as did Kashin, that the population be removed from the affected area.

It is currently believed that the Kashin-Beck syndrome is the result of an infestation of the grain grown in the affected area by the parasitic fungus *Fusarium sporotrichiella*.[67] Symptoms resembling the Kashin-Beck syndrome were observed in rats fed grain contaminated by the fungus, as well as various putrefaction products of proteins, such as tyramine, cadaverine, and putrescine. It is believed that the fungus elaborates an enzyme or enzymes that cause the putrefaction of wheat protein, and the putrefaction products, in turn, are responsible for the pathology observed in Kashin-Beck syndrome. Treatment now consists of substituting "imported" foods for those produced locally.

One of the great successes of modern biochemistry is the identification of a number of inherited diseases as aberrations of enzymatic reactions. Among such diseases, are the various porphyrias, the glycogen storage diseases, phenylketonuria, and a number of other inborn errors of amino acid metabolism. Alcaptonuria, a defect in the metabolism of tyrosine, has been known for a long time, and is characterized by the excretion of an abnormal substance in the urine, which turns black upon exposure of the urine to air. The nature of this substance was elucidated in 1891 by M. Volkov from St. Petersburg, who was working in Baumann's laboratory in Germany at that time. He showed that the unknown substance was homogentisic acid.[68] Contrary to modern view, Volkov felt that the homogentisic acid was not a metabolic product of tyrosine, but was, instead, produced by microorganisms somewhere in the patient's system. Intestinal microorganisms were apparently found not to be responsible.

CONCLUSION

It would be interesting to speculate as to who were the most distinguished biochemists in Russia of the Tsarist era. In view of modern biochemical knowledge, the most progressive investigators were probably Lunin, Gulevich, Danilevsky, and Alexander Schmidt. These would be closely followed by the synthesizers such as Lebedev[16] and Cyon. The experiments of Anichkov and Khalatov were probably most relevant to today's medical interests, though these are generally associated with pathology rather than biochemistry. The men who probably did most to promote interest in biochemistry in Russia were Nencki, Carl Schmidt, and, after World War I, A. N. Bach. Nencki was best known abroad.[48] By 1913, there were chairs of biochemistry in all of Russia's medical schools, and in several veterinary colleges. Biochemical institutes were associated with the Moscow, Kazan, and Kharkov universities.[69] It is thus quite apparent that biochemistry had found its way into Russia well before the First World War, and that its practitioners in Russia were important pioneers in this modern field of the natural sciences.

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BOOK REVIEW

MEDICAL MALPRACTICE LAW by Angela Roddey Holder. 575 pp., New York, John Wiley and Sons, 1975. $22.50

This book deals with the legal principles inherent in physician-patient relationships, citing over 1,000 cases including virtually every known type of malpractice action. Misdiagnosis, incorrect or inadequate therapy, injuries from therapeutic agents or equipment, consent to treatment, the defense to malpractice action, and the termination of the physician-patient relationship are the areas covered. Of the many cases cited, three that I found interesting are as follows. Under the topic "Contractual Relations" was a case involving a medical school professor who examined a clinic patient prior to selecting a few patients to participate in a lecture series that he gave at the medical school. One of the patients he saw had an injured knee. He saw her for only a moment and did not use her in his lecture, but in an aside to the resident he advised that her leg be amputated. He never actually treated the patient, never saw her beyond that momentary interlude, but was named as one of the parties found liable by the court.

Under the chapter "Injuries From Therapeutic Agents," the section on drugs is quite frightening from the standpoint of the physician's liability. In one case, "a patient had, in sequence, two D&C's, a hysterectomy and an operation to relieve an intestinal obstruction. During all the periods of hospitalization, she received large doses of both morphine and Demerol. After her release from the hospital, the defendant prescribed morphine for self-injection at any time she requested it. The physician was liable not only for actual damages, but for punitive, or extra, 'punishment' damages for her addiction." In the chapter "Consent to Treatment," it was emphasized that when a physician treats a patient by a method which entails certain risks. and alternate treatment exists, he is obliged to advise the patient of that alternative. Failure to explain an alternative, if one exists, may be construed as negligence, even if the physician thinks it is less desirable. "A patient with a prostate disorder was admitted to the hospital for surgery. The surgeon did not advise him that the operation planned would inevitably sterilize him. He also did not advise him that an alternative surgical method was available which carried with it a higher risk of infection. The patient underwent the operation which sterilized him. He sued the physician when he discovered later that he was sterile. The court upheld the jury's award of damages to the patient. The court held that, in the absence of an emergency, the patient should have been advised of all available alternatives and that he had a right to make up his own mind as to the alternative which he preferred."

In summary, this is an extensively well-documented book covering the fundamentals of medical malpractice law.

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ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Anesthesiology

El-Naggar M: *The use of a small endotracheal tube in bronchoscopy. Br J Anaesth 47:390, 1975*

The use of the standard 4-mm cuffed endotracheal tube to deliver manually or mechanically interrupted jets of oxygen or nitrous oxide and oxygen into the trachea allowed bronchoscopy and other related procedures to be performed with advantage to the patient, the surgeon and the anaesthetist. This paper describes the equipment used and the advantages obtained.

Hatano S, Keane D, Wade MA, Sadove MS: *Diazepam-pentazocine anaesthesia for cardiovascular surgery. Canad Anaesth Soc J 21:586, 1974*

We have reported a series of 320 patients anaesthetized for operations with cardiopulmonary bypass using a combination of diazepam and pentazocine supplemented with nitrous oxide and oxygen. We found it to be a satisfactory technique for the anaesthetic management of patients with poor cardiac reserve. Ease of induction and maintenance were noteworthy with this technique, with minimal side effects during anaesthesia and in the post-operative period. Although the use of pentazocine in large dosage has not been officially approved, we feel that use of a combination of diazepam and pentazocine in the technique we have described has many advantages in anaesthesia for operations with cardiopulmonary bypass.

Johnson B, Thomason R, Pallares V, Sadove MS: *Autonomic hyperreflexia: a review. Milit Med 140:345, 1975*

The symptoms. signs, morbidity, pathophysiology, and therapy of autonomic hyperreflexia have been reviewed. An alternate pathophysiologic mechanism has been proposed. A strong plea is made for those managing cord injury patients to be aware of this syndrome and its consequences, and to realistically treat it by the means outlined in an effort to provide better care for this unfortunate group of susceptible individuals.

Liu TH, Sadove MS: *Scalp needle therapy-acupuncture treatment for central nervous system disorders. Am J Chin Med 2:261, 1974*

This article reviews the history and clinical applications of the recently developed technique of acupuncture on the scalp or "scalp needle therapy" for the treatment of central nervous system diseases and syndromes of various types resulting from chorea, parkinsonism, peripheral vascular injury, encephalitis and other conditions. The authors discuss several cases treated in this manner and report that it has resulted in significant clinical improvement in a small number of patients.

Okazaki K, Sadove MS, Kim SI, Lee MH, Cheng D: *Ryodoraku therapy for migraine headache. Am J Chin Med 3:61, 1975*

The authors discuss Ryodoraku electric acupuncture therapy in detail in Part 1. The results of the treatment of migraine headache (20 cases) by Ryodoraku therapy were investigated in part II. In the present study 15 out of 20 patients achieved good-to-excellent responses to this type of therapy.

Cardiology

Kessler HA, Liebson PR, Mattenheimer H, Adams EC, Jr.: *Acute myocardial infarction diagnosed by myoglobinuria. Arch Intern Med 135:1181, 1975*

Myoglobin can be found in excess in the urines of some patients with acute myocardial infarction. To test the specificity of this finding, urine specimens were analyzed from 39 patients with provisional diagnosis of myocardial infarction by means of a hemagglutination-inhibition technique with prepared antisera to monkey myoglobin.

Of 24 patients with subsequently documented myocardial infarction, 15 had at least one positive determination. None of the 15 patients without infarction had positive tests. Ten of 13 patients with infarction studied within 24 hours of the initial event had positive reactions. The percentage of positive reactions in the infarct group decreased sharply after the first 24 hours.

This technique may be of value in rapid screening of patients with possible acute myocardial infarction during early stage of symptoms.

Susmano A, Muenster JJ, Javid H, Carleton RA: *Coronary arteritis in rheumatoid arthritis. Arch Intern Med 132:241, 1973*

Rheumatoid arthritis may be accompanied by cardiovascular lesions. This may include valvular, myocardial and pericardial involvement as well as systemic arteritis. Arteritis of the coronary vessels (capable of producing acute myocardial infarction) has been found at autopsy in about 20 percent of the cases, but rarely has this complication been diagnosed during life.

This paper describes the clinical and coronary arteriographic features of a patient with rheumatoid coronary arteritis in whom angina pectoris was treated by saphenous vein aortocoronary bypass.

Cardiovascular Thoracic Surgery

Chawla SK, Najafi H, Ing TS, Dye WS, Javid H, Hunter JA, Goldin MD, Serry C: *Acute renal failure complicating ruptured abdominal aortic aneurysm. Arch Surg 110: 521, 1975*

Ruptured abdominal aortic aneurysm complicated by renal failure is associated with a mortality greater than 90 percent. Aggressive management, which included the early use of hemodialysis, was employed. Between 1970 and 1973, a total of 43 patients had surgery for proved ruptured abdominal aortic aneurysm. Fourteen patients developed acute and fixed renal failure. Nine of these 14 patients had undergone hemodialysis with treatments beginning as early as the second postoperative day and lasting as long as ten weeks. There were six survivors, with a mortality of 33 percent. This represents an im-

provement in survival compared with our earlier experience where the mortality in this type of patient was 93 percent. Early use of hemodialysis in the postoperative management of patients with acute renal failure complicating ruptured abdominal aortic aneurysm is recommended.

Jensik RJ, Faber LP, Brown CM, Kittle CF: *Bronchoplastic and conservative resectional procedures for bronchial adenoma. J Thorac Cardiovasc Surg 68:556, 1974*

The low-grade malignant potential of bronchial adenoma has been widely reported in the medical literature. We believe that the most conservative types of resection are adequate for most patients with this disease. Our experience with carcinoid tumors of the bronchus reinforces this belief. During the past 18 years we have treated 33 patients with the disease, only 10 of whom required conventional lobectomy or pneumonectomy. The other 70 percent were treated by bronchoplasty or more conservative resectional procedures. The recurrence rate in this series was extremely low, and the survival rate, calculated by the life-table method, was 86 percent at five years.

Immunology

Schutte M, DiCamelli R, Murphy P, Sadove M, Gewurz H: *Effect of anesthesia, surgery and inflammation upon host defense mechanisms: I. Effects upon the complement system. Int Arch Allergy Appl Immunol 48:706, 1975*

Complement protein levels and C7 hemolytic activity were measured in four individuals following anesthesia and surgery, and in a group of 20 patients with inflammatory diseases. Seven of the eight complement components studied characteristically were elevated, most dramatically C1s and C3PA. Elevation of C1s often was greater than elevation of C1q, displaying an independent variation of C1s and C1q in both postoperative and inflammatory disease patient groups. The major increases of C components were seen subsequent to the peak C-reactive protein response, as was the occurrence of the 'reactor state,' a propensity to formation of C56 which surprisingly was associated with increased levels of C7. Levels of properdin frequently were reduced postoperatively. It is concluded that multiple complement components, with the notable exception of properdin, respond as acute phase reactants which are elevated and changed in proportion postoperatively and during inflammatory disease.

Siegel J, Osmand AP, Wilson MF, Gewurz H: *Interactions of C-reactive protein with the complement system: II C-reactive protein-mediated consumption of complement by poly-l-lysine polymers and other polycations. J Exp Med 142:709, 1975*

Cationic homopolymers of poly-l-lysine were found to activate complement (C) via C-reactive protein (CRP) and deplete C3 and C5 as well as early-acting C components. Maximum C consumption was obtained with polymers of 2,000-8,000 daltons; polymers of 1,700, 11,000, and 23,000 daltons were intermediate in reactivity, while L-lysine, lysyl-l-lysine, tetra-l-lysine, and polymers of 70,000 to 400,000 daltons lacked significant C-consuming activity. Naturally occurring polycations which consumed C in the presence of CRP included myelin basic proteins, cationic proteins of rabbit leukocytes, and both lysine- and arginine-rich histones. Poly-L-arginine polymers of 17,000 but not 65,000 daltons also were C-consuming. Polycations without such reactivity included poly-L-ornithine (5,000 and 165,000 daltons), egg white and human lysozymes, and Polybrene. The polycations which failed to induce C consumption via CRP, inhibited its consumption by both active polycations and by C-polysaccharide (CPS). The relative

inhibitory capacity of phosphorylcholine and polycations in CPS- and polycations-CRP systems was consistent with the concept that phosphate esters and polycations react at the same or an overlapping combining site. The ability of certain polycations to activate C via CRP increases the potential for initiation of host reactions via C. The capacity of other polycations to inhibit C activation via CRP introduces a potential for physiologic or pharmacologic manipulation. These considerations would seem to expand the potential role of CRP in the initiation and modulation of the inflammatory response.

Microbiology

Carrico RJ, Beagle R, Usategui-Gomez M, Deinhardt F: *A radioimmunoassay specific for the antigenic determinants exposed by pepsin digestion of human immunoglobulin G. Immunochemistry 11:573, 1974*

$F(ab')_2$ fragment prepared by pepsin digestion of human IgG was used to raise antibody in goats. Adsorption of this antibody with whole IgG bound to Sepharose® yielded an antibody preparation which reacted specifically with the pepsin site of $F(ab')_2$. The adsorbed antibody was employed in a radioimmunoassay which was highly specific for the pepsin site. In measurements conducted on sera, the radioimmunoassay did not distinguish between $F(ab')_2$-like protein and naturally occurring antibody to the pepsin site. Results of assays showed the presence of pepsin site activity in all sera examined. In order to determine whether antigen or antibody was present, some of the sera were adsorbed with $F(ab')_2$ bound to Sepharose® and assays conducted after this treatment showed that the pepsin site activity was decreased. Thus, antibody to the pepsin site was present in these sera. Essentially the same levels of pepsin site activity were found in sera from normal individuals and from patients with cancer. About 5 to 7 percent of the sera in both groups had activities notably higher than the average level. Pepsin site activities in sera from patients with rheumatoid arthritis and lupus erythematosus were lower than the average level found in normal sera. To determine if this difference is significant, assays must be conducted on sera from more patients with these diseases.

Deinhardt F: *Introduction to virus-caused cancer: type C virus. Cancer 34:1363, 1974*

Ribonucleic acid-containing, C-type viruses have been isolated from almost every class of animals; they transform cells *in vitro* and cause leukemias and sarcomas *in vivo*. Although these viruses are structurally, biochemically, and biologically similar, they can be distinguished from each other by individually characteristic nucleic acids and antigens. They are usually transmitted vertically; evidence of their presence, in the form of nucleic acid sequences homologous to viral nucleic acid sequences, may be detected in normal cells. Many C-type viruses are defective; helperviruses are needed for full viral genome expression. Human C-type leukemia or sarcoma viruses have not been identified with certainty yet, although their existence can be predicted from the results of experimental animal studies and immunologic, ultrastructural, and biochemical analyses of human tumors.

Deinhardt F, Wolfe L, Falk L, Johnson T, Johnson D, Massey R: *Immunological control of virus-induced tumors in primates. Comp Leuk Res 1973, Leukemogenesis, Ed. Y. Ito and R. M. Dutcher, Univ. of Tokyo Press, Tokyo/Karger, Basel, 1975, pp. 639-648*

Cells infected by oncogenic viruses may transform, may develop a latent carrier state, or may be destroyed, but understanding of the control of the results of infection is incomplete. Even if cells transform, ultimate development of a tumor may be immunologically controlled. For example, cells of some marmoset species transform after infection with RNA tumor viruses, and animals react to the transformed cells with cell-mediated and humoral immune responses. Both virus-specific and cross-reacting cell membrane antigens have been demonstrated. Immune deficiency accelerates tumor growth or causes recurrence of a regressing tumor. In contrast, certain simian herpesviruses *(Herpesvirus saimiri,* HVS and *Herpesvirus ateles,* HVA), which cause no or minor disease in their natural hosts, induce lymphomas or lymphoblastic leukemias in other primate species. The immune response of the natural host species to HVS is greater than that of animals developing malignancies after experimental infection. HVS and HVA share many properties with Epstein-Barr virus (EBV) of man, including antigens appearing early and late during infection and their related antibody responses, but no evidence exists that they induce malignancies in their natural hosts. However, if induction is as infrequent as that with EBV and Burkitt's lymphoma (BL), we have not observed sufficient numbers of squirrel or spider monkeys to have seen a BL-like tumor. Interference with the immune systems of animals carrying HVS or HVA may induce tumor development, and clarify our understanding of the relationship between EBV and BL.

Fischer RG, Falk LA, Rytter R, Burton GJ, Luecke DH, Deinhardt F: *Herpesvirus saimiri: viability in four species of hematophagous insects and attempted insect transmission to marmosets. J Natl Can Inst 52:1477, 1974*

Viability of the *Herpesvirus saimiri* (HVS) genome was investigated in the stablefly, *Stomoxys calcitrans,* the cat flea, *Ctenocephalides felis,* and the mosquitoes, *Aedes aegypti* and *Anopheles quadrimaculatus.* The infected donors were adult white-lipped (WL) *(Saquinus fusciollis)* and cotton-topped (CT) *(S. oedipus)* marmosets whose blood had HVS in the genome or repressed state in most lymphocytes. By the cocultivation of the dissected gut contents or ground-up whole insects on permissive vero cells. infectious virus was demonstrated immediately and at six hours after ingestion in all four insect species, but could not be recovered at 0.5, 1, 2, 4, 6, 8, 11, and 12 days post-feeding. Mechanical transmission from infected to noninfected CT and WL marmosets was studied by interrupted feeding procedures, with *S. calcitrans, A. aegypti,* and the cone-nose bug, *Rhodnius prolixus.* Eight recipient marmosets were kept in Horsfall-type isolation units and observed for 8 to 13 months. No evidence of disease or hematologic abnormalities were found, whereas two positive controls needle-inoculated with whole blood from the infected donors developed disease and died at 42 and 57 days post-inoculation.

Froesner GG, Peterson DA, Holmes AW, Deinhardt FW: *Prevalence of antibody to hepatitis B surface antigen in various populations. Infect Immun 11:732, 1975*

Sera from individuals with different degrees of exposure to the agent of hepatitis B were tested for antibodies to hepatitis B surface antigen (anti-HB_S) by passive hemagglutination and for hepatitis B surface antigen (HB_S Ag) by radioimmunoassay and immunoelectroosmophoresis. In a plasma fractionation plant, anti-HB was detected in 82 percent of workers processing human plasma and 3.3 percent were healthy carriers

of antigen. Fifty-six percent of the workers having only casual contact with plasma processing exhibited anti-HB_S and 24 percent of workers with no contact had anti-HB_S, yet HB_S Ag was not found in either of these two groups of workers. A similar correlation was shown in hospital personnel; 31 percent of employees with direct contact to serum specimens and only 8 percent without direct contact had anti-HB_S. The frequency of HB_S Ag (0.8 percent in patients with disorders not involving the liver; 49.8 percent in patients tentatively diagnosed as viral hepatitis) and anti-HB_S (14.5 percent to 28.5 percent, respectively) in selected groups of hospitalized patients varied greatly. In 508 paid blood donors, anti-HB_S was present in 19.9 percent, whereas it was present in only 6.6 percent of 1,146 volunteer donors. These data demonstrate a correlation between frequency of exposure to human blood or blood products and the prevalence of anti-HB_S.

Hoekstra J, Deinhardt F: *Simian sarcoma and feline leukemia virus antigens: isolation of species- and interspecies-specific proteins. Intervirology 2:222, 1973/74*

Feline leukemia virus (FeLV) and simian sarcoma virus (SSV-1/SSAV-1) each contain six proteins which were separated by gel filtration in 6 m guanidine hydrochloride. The major protein both of FeLV and of SSV-1/SSAV-1 has species and interspecies antigenic determinants, and both viruses contain additional viral proteins with distinct species-specific antigenic determinant. The interspecies antigens of two primate C-type viruses (SSV-1/SSAV-1 and gibbon ape lymphoma virus) appear to be nearly identical and react only weakly with the corresponding antigens of lower animals.

Holmes AW, Cross GF, Peterson DA, Deinhardt F: *Differentiation of HAAg and HBAg: identification of the hepatitis A virus. In: Transmissible Disease & Blood Transfusion. Tibor J. Greenwalt, M.D. & Graham A. Jamieson, Ph.D., D.Sc., editors, New York, 1975, Grune & Stratton, Inc., pp. 33-42*

Marmosets have been shown to be reliable animals in which to study human hepatitis type A. Biochemical and morphologic changes in infected animals are similar to those seen in man. Convalescent serum from humans will neutralize infectivity when tested in the marmoset model. Marmosets will, in the acute phase, excrete an antigen in the stool that has been shown previously to be present in acute-phase stool of patients with hepatitis A. An antigen-positive human stool has been used to infect marmosets, and the animals from that experiment that developed hepatitis excreted the antigen in their stools in the acute phase of their disease.

Patterson RL, Peterson DA, Deinhardt F, Howard F: *Rubella and rheumatoid arthritis: hyaluronic acid and susceptibility of cultured rheumatoid synovial cells to viruses. Proc Soc Exp Biol Med 149:594, 1975*

Synovial cell lines were established from patients with rheumatoid arthritis (RA) and from normal human embryos. High levels of hyaluronic acid (HA) were produced by some RA cell lines, some of which were partially or completely resistant to infection with Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), and rubella virus (RV). Normal fetal synovial cells lines were susceptible to NDV, VSV, and RV. Infection with virus became possible after treatment of RA cells with hyaluronidase to depolymerize HA, and HA prevented infection of normal synovial cells with VSV. These results provide evidence that HA and not chronic or latent viral infection is responsible for the lack of susceptibility of RA synovial cells to certain viruses.

Schauf V, Falk L, Deinhardt F: *Effect of Bacillus Calmette-Guérin immunization in marmosets infected experimentally with* Herpesvirus saimiri. *J Natl Cancer Inst 54:721, 1975*

Eight white-lipped marmosets immunized with BCG and three sham-immunized marmosets were studied after inoculation with *Herpesvirus saimiri* (HVS). BCG immunization had no significant influence on the incidence of infection by HVS, incidence of fatal malignant lymphoma, time of leukemia onset, development or titer of HVS antibodies, or average survival time. One BCG-immunized, HVS-infected marmoset failed to develop malignant lymphoma, whereas the remaining 10 HVS-infected marmosets died of malignant lymphoma. Prolonged survival occurred also in one marmoset immunized with BCG 100 days after HVS inoculation. The development and disappearance of lymphocyte reactivity to tuberculin were followed in four BCG-immunized marmosets.

Schauf V, Kruse R, Deinhardt F: *BCG immunization in marmosets. J Med Prim 3:315, 1974*

Clinical and immune responses of marmosets to BCG immunization were studied. Ten of 13 animals responded to BCG immunization by developing lymphocyte reactivity to purified protein derivative (PPD), while the lymphocytes of only three of 64 unimmunized animals showed reactivity to PPD.

Schauf V, Massey R, Deinhardt F, Kruse R: *Immunization of Rous sarcoma virus-inoculated marmosets with BCG and transformed allogeneic cells. J Natl Cancer Inst 54:151, 1975*

The effects of specific immunotherapy with allogeneic cells transformed by Schmidt-Ruppin Rous sarcoma virus (SR-RSV), of treatment with BCG, and of surgery on the growth of SR-RSV-induced sarcomas in white-lipped marmosets were studied. Tumor incidence, tumor progression, and survival did not differ between control and treated animals. Animals immunized with BCG developed lymphocyte reactivity to tuberculin, which remained until the animals died. BCG was isolated from the spleen of one tumor-bearing animal.

Tanaka A, Nonoyama M: *Latent DNA of Epstein-Barr virus: separation from high-molecular-weight cell DNA in a neutral glycerol gradient. Proc Nat Acad Sci 71:4658, 1974*

Epstein-Barr virus DNA in virus non-productive cells was separated from high-molecular-weight cell DNA by sedimentation through a neutral glycerol gradient after gentle lysis of cells by Pronase and Sarkosyl. The isolated Epstein-Barr virus DNA had a density of 1.716 to 1.717 g/cm^3 in CsCl equilibrium centrifugation, which is very close to the virus DNA density of 1.718 g/cm^3. The results indicated that the majority of Epstein-Barr virus DNA in virus nonproductive cells is not covalently integrated into high-molecular-weight cell DNA.

Ophthalmology

Hughes WF, Coogan PS: *Pathology of the pigment epithelium and retina in rabbits poisoned with lead. Am J Pathol 77:237, 1974*

Multifocal lesions of the retinal pigment epithelium were observed in rabbits fed a diet containing 0.5 percent lead subacetate for periods of up to two years. Groups of pigment epithelial cells became congested with a lipofuscin pigment which was apparently derived from phagosomes of rod outer segments. Lipofuscin granules displaced melanin granules from the apical surface of the retinal pigment epithelial cells and resulted in conspicuous brown pigmentation of these cells in albino animals. Migration of macrophages and pigment epithelial cells into the subretinal space was common in affected areas. This pathology was not observed in the pigment epithelium of the ora serrata, or ciliary body. At the latest time periods, the abnormal lipofuscin pigmentation subsided, and degeneration of photoreceptors occurred. The pathogenesis of the lesions is discussed.

Hughes WF, LaVelle A: *On the synaptogenic sequence in the chick retina. Anat Rec 179:297, 1974*

Study of the developing chick retina with the electron microscope revealed that dyad ribbon synapses begin to form in the inner plexiform layer before synaptic ribbons begin to appear in photoreceptor terminals of the outer plexiform layer. This centrifugal (inner to outer) sequence of synaptogenesis in the predominantly cone retina of the chick differs from the centripetal sequence that has been reported for the predominantly rod retinas of the mouse and rat. This difference does not favor the hypothesis, suggested by others, that the photoreceptor may influence the maturation of inner retinal elements. The different patterns of synaptogenesis are discussed briefly with reference to anatomical differences between the retinas of different species.

Orthopedics

Guenther HL, Sorgente N, Guenther HE, Eisenstein R, Kuettner KE: *Lysozyme in preosseous cartilage; VI. Purification, characterization and localization of mammalian cartilage lysozyme. Biochim Biophys Acta 372:321, 1974*

Lysozyme (mucopeptide-N-acetylmuramylhydrolase, EC 3.2.1.17) is present in mammalian cartilage. Lysozyme was isolated and purified from bovine and canine cartilage and from dog serum using various chromatographic steps and affinity chromatography on carboxymethylated chitin. Amino acid analysis of bovine cartilage lysozyme showed that it is similar to other mammalian lysozymes. Anti-canine lysozyme antibodies cross-react with calf lysozyme, but not with hen egg white or embryonic chick cartilage lysozyme. In the epiphyseal plate of the dog, 90-μm sections were analyzed for lysozyme, and it was found that in the hypertrophic zone its concentration is approximately six times higher than it is in the resting zone. Using immunocytochemical techniques at the electronmicroscopic level, lysozyme in the epiphyseal plate of the dog was localized extracellularly, mainly in the immediate vicinity of the chondrocytes, the territorial matrix.

Kuettner KE, Sorgente N, Croxen RL, Howell DS, Pita JC: *Lysozyme in preosseous cartilage; VII. Evidence for physiological role of lysozyme in normal endochondral calcification. Biochim Biophys Acta 372:335, 1974*

Previous work demonstrated that micropuncture aspirates from rat epiphyseal plate cartilage contain a nucleating agent for $Ca_3(PO_4)_2$ mineral growth, and that the nucleation is inhibited by proteoglycan aggregates. In this report data are described which show that mammalian lysozyme inactivates the inhibition. When micropuncture aspirates are incubated *in vitro* with mammalian lysozyme, a rapid, spontaneous initiation of mineral growth occurs. Incubation of proteoglycan aggregate preparations in the presence of cartilage lysozyme, but not hen egg white lysozyme, causes a marked decrease of the sedimentation coefficients of the proteoglycans, usually to values close to those obtained with proteoglycan monomer preparations. The inhibition of this effect of mammalian lysozyme by a specific inhibitor of this enzyme tri(N-acetyl-D-glucosamine) suggests that it may be enzymatic in nature.

Sorgente N, Kuettner KE, Soble LW, Eisenstein R: *The resistance of certain tissues to invasion. II. Evidence for extractable factors in cartilage which inhibit invasion by vascularized mesenchyme. Lab Invest 32:217, 1975*

If hyaline cartilage is explanted to the chick chorioallantoic membrane, it resists invasion by vascularized mesenchyme of the host. This resistance is diminished if the tissue is extracted with relatively low concentraions (1.0M) of guanidine HCl. The extracts contain antiproteolytic activity. This molarity of guanidine HCl extracts only small amounts of the major structural components of cartilage extracellular matrix. It, therefore, seems reasonable to suggest that hyaline cartilage is both avascular and resistant to invasion because it contains extractable inhibitors or invasion, perhaps in the form of proteinase inhibitors.

Wezeman FH, Kuettner KE: *Selective histochemical staining of cartilage matrix by Rivanol.® Anat Rec 180:481, 1974*

Rivanol,® a fluorescent ethoxy derivative of acridine, interacts at different pH's with both glycosaminoglycans and proteins. The present study utilizes the specific interaction of Rivanol® with acidic substances of the ground substance for histochemical studies of the cartilage matrix. This stain was applied to newborn mouse epiphyseal cartilages which were either unextracted or dissociatively extracted by graded concentrations of guanidinium chloride (GuHCl) from 0.5 to 3.0 m for four days at 25° C. Routinely prepared sections were then stained (0.1 percent solution) for two minutes at pH's ranging from 2.2 to 11.2. Stainability of the interterritorial matrix as well as the inner halo zone and outer corona zone of the lacunar matrix varied with pH. Whereas the interterritorial matrix decreased in stainability with rising pH, the halo and corona persisted in stainability up to pH 10.7. Dissociative extractions using GuHCl revealed the unextractable nature of the inner halo zone as well as the extractable nature of the corona above 1.0 m GuHCl concentration. Anionic sites on polyelectrolytes such as glycosaminoglycans are known to stoichiometrically bind many cationic dyes. The precise localization of stain-reactive glycosaminoglycans or proteoglycans in the region of the perichondrocytic matrix by Rivanol® supports prior observations using other cationic stains. Our data demonstrates that Rivanol® enables one to visualize the unique perichondrocytic matrix which may be interpreted to be both chemically and morphologically a "matrix within a matrix."

Otolaryngology

Andrews AH, Moss HW: *Experiences with the carbon dioxide laser in the larynx. Ann Otol Rhinol Laryngol 83:462, 1974*

Techniques for the application of the carbon dioxide laser to laryngeal surgery are outlined and include operative and anesthetic management. Laser surgery teaches improved surgical judgment in and application of conventional procedures. The lack of trauma, the degree of precision and the speed of healing as seen in one year of experience indicate the results of laser surgery to be superior to conventional techniques in the treatment of papilloma, polyps, polypoid degeneration, hyperkeratosis and carcinoma *in situ.* A family of laryngeal retractors is introduced whose utilization with intralaryngeal mirrors makes essentially all areas of the larynx accessible for lasing.

Friedberg SA, Azem K, Wallner LJ: *Tracheostome maintenance by obturator for prolonged or future need. Ann Otol Rhinol Laryngol 83:520, 1974*

Maintenance of a tracheostome by obturator is a valuable adjunct in airway-related problems of long duration. Obturators have hitherto been used principally for infants and children during decannulation efforts. Synthetic obturators are useful in adults who may not require a conventional tracheal cannula for breathing for prolonged periods or when the probability exists of need for a repeat tracheostomy in the future. Patients with chronic lung disease, vocal cord paralysis with polypoid corditis, lupus erythematosus, pemphigus, myasthenia gravis and head and neck cancer have had their tracheostomes maintained by synthetic obturators during symptom-free periods. It is simple to substitute conventional tracheal cannulas or cuff tubes when needed. Synthetic obturators are simply made, inexpensive, easily cleaned, cause no tissue reaction and permit speech without finger occlusion.

Pediatrics

Haddy TB, Jurkowski C, Brody H, Kallen DJ, Czajka-Narins DM: *Iron deficiency with and without anemia in infants and children. Am J Dis Child 128, 1974*

Among 109 infants and children from four months to five years of age from low-income families, hemoglobin concentrations lower than 11 gm/100 ml and hematocrit values lower than 33 percent indicated iron-deficiency anemia, but higher levels did not exclude deficiency. Seventy-nine of the 109 were studied during a three-month screening program. Of these, one third had iron deficiency. When only children under two years of age were considered, however, the incidence was slightly more than 50 percent. A 24-hour food intake history correlated well with the presence or absence of iron deficiency and was a useful instrument for evaluating the adequacy of dietary iron in this population.

Narins DC, Hirsch J: *Supplementary feeding during the preweaning period: effect on carcass composition and adipose tissue cellularity of the rat. Biol Neonate 25:176, 1974*

The present study was undertaken to determine if a model system could be established in the rat to produce hypercellularity of adipose tissue by overfeeding the preweaning

period comparable to the eating pattern which has been suggested as contributing to one form of human obesity. To produce the hypercellularity, rat pups were given four supplementary feedings daily during the preweaning period. Supplementation resulted in significant differences in carcass composition, early in percent of fat (14 and 20 days) and later, in percent of protein (40 and 105 days of age). Although there was no significant difference in cell number between the supplemented and control rats, there were significant differences in cell size, the supplemented animals having larger cells at 40 and 105 days of age. The pattern of cellularity produced by supplementation was, therefore, different from that produced by other techniques of early overnutrition.

Therapeutic Radiology

Hendrickson FR: *Radiation therapy of metastatic tumors. Semin Oncol 2:43, 1975*

In the management of patients with metastatic cancer to the brain there are selected situations in which surgical extirpation is appropriate. For patients with severe neurologic findings the concomitant use of corticosteroids for prompt relief of symptoms coupled with radiotherapy to the contents of the calvarium has a high probability of temporary relief of symptoms. For patients with less severe neurologic findings there appears to be no advantage to any combination of agents over radiotherapy alone. Overall management will be dictated by other sites of metastases and the general status of the patient. The severe distressing symptoms of convulsions and headache can be improved or totally eradicated in more than 85 percent of the patients. Impaired mentation and motor loss can be partially or completely recovered in more than 70 percent of the patients. While the length of life may not be modified, radiotherapy significantly improves the quality of survival.

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